

THE AMERICAN ACADEMY OF FORENSIC SCIENCES

PROCEEDINGS

# A RESPONSIVE ACADEMY

MEETING AND SURPASSING THE CHALLENGES OF A  
MODERN FORENSIC SCIENCE WORLD



**AAAFS**  
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### ***PROCEEDINGS***

of the American Academy of Forensic Sciences 74<sup>th</sup> Annual Scientific Conference

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 Conference. These include various branches of the forensic sciences such as anthropology, criminalistics, digital evidence, engineering, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

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# ***PROCEEDINGS***

**of the American Academy of Forensic Sciences**

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### S1 Interdisciplinary Symposium: Problems of Today and Solutions for Tomorrow

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of current challenges in multiple forensic science disciplines, death investigations, and in the courts.

**Impact Statement:** This presentation will impact the forensic sciences community by offering various perspectives regarding current issues and possible future solutions.

This session will present various perspectives as they relate to forensic science, medicine, and the courts including: workload and workforce issues in forensic pathology and their impact on death investigations; the analytical challenges presented by the rapid development of illicit novel psychoactive substances; why standards created by the Academy Standards Board (ASB) are crucial to forensic science; and how pattern recognition discipline validity challenges are being addressed. The symposium will also discuss why courts are both reluctant to recognize challenges to previously accepted forensic science techniques and resistant to admitting evolved techniques.

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**Forensic Science Disciplines, Death Investigation, Court**

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### S2 Young Forensic Scientist Forum (YFSF) Special Session: Meeting and Overcoming Challenges Faced by Young Forensic Scientists

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**Learning Overview:** The YFSF will aim to bring to light some of these challenges and more importantly, highlight how the members and attendees of our very own American Academy of Forensic Science met these challenges head on, rose to the occasion and circumvented these challenges in order to get to their current positions.

**Impact Statement:** The impact of this Special Session will focus mostly on, but not exclusively on, the younger forensic scientists who are still in the process of trying to get to their "dream job". We say not exclusively because the age of the person will never dictate their entrance into a new career in forensics, therefore anyone and everyone can and will benefit from the information provided in this Special Session.

This session is designed from the ground up and tailored to the Young Forensic Scientists Forum attendees as well as any members of the AAFS who are or were experiencing difficulties in the transition from Student to Professional. So many hurdles are present in this journey, many of which young scientists are completely unprepared for: building and updating resumes, job interviewing skills and techniques, proper and efficient networking, and all the mistakes, difficulties and challenges in between.

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#### YFSF, Forensics, Special Session

### S3 Tale of the Tape—The Media’s Impact on Forensic Science Cases

*J.C.U. Downs, MD\*, forensX, LLC, Charleston, SC; Tanisha Henson, JD\*, Maricopa County District Attorney’s Office, Phoenix, AZ; Barry Fisher, MBA, MS\*, NIJ, Indio, CA; John Fudenberg, MBA\*, IACME, Las Vegas, NV; Judy Melink, MD\*, PathologyExpert, Inc., San Francisco, CA; Bobbi Jo O’Neal, RN\*, Charleston County, North Charleston, SC; Greg Ramey, MPA\*, Lafayette, GA; Edwin P. Stevens, APS Films LLC, Glendale, CA*

**Learning Overview:** Attendees can expect to learn about the impact that media can and does have on forensic casework at multiple levels and how to successfully engage with journalists while maintaining objectivity in casework.

**Impact Statement:** This presentation will impact the forensic science community by showing documented pitfalls and successes in various media and forensic science professionals in handling casework and suggest strategies for future improvement in the flow of information while protecting necessary information.

Forensics professionals are keenly aware of the impact their services have on cases. Part and parcel of this is intense public interest in and scrutiny of casework. One need only do a brief internet search to find over 1.5 million hits for “case of the century,” including references to the accompanying media frenzies associated therewith. In many settings, the players—forensics experts and the media—are placed at seeming odds with each other, in a real or perceived effort to control information flow. Comfort comes in knowing that the crucible of second-guessing burns hot—but only until the next such case comes along. As this cycle of investigation-analysis-court-critique-resolution repeats, lessons are available for those willing to learn from their forebearers. As Voltaire asked, “Is there anyone so wise as to learn by the experience of others?”

All cases begin at the scene, where experience teaches many cases are won or lost based upon how a scene is processed and how an initial investigation commences. Los Angeles County, CA, is an enormous jurisdiction and no stranger to high-profile cases, including O.J. Simpson, Phil Spector, The Night Stalker, and too many others to count. A discussion of lessons learned from decades of leading the forensic laboratory analysis of such high profile cases provides valuable insights into the “dos” and “don’ts” of casework.

High-interest cases, far from being limited to major jurisdictions, can literally occur anywhere and at any time. Case in point, 20 years ago a sleepy northwest Georgia community of just over 60,000 people became home to a macabre nightmare of a case—an originally unknown number of human remains (later tallied at 339) was found literally dumped in and around the grounds of a family compound surrounding an operating crematory. Nothing similar on such a scale had been encountered before or since. The primary investigators had to deal with not only the casework to be done in order to identify the deceased and to prove criminal charges, but had to simultaneously inform the public while preventing the media from interfering with investigators’ duties. Issues encountered along the way proved challenging but a (partial, at least) successful case resolution followed, thanks to good public relations anchoring good detective work.

Many jurisdictions conduct exhaustive preparatory exercises in order to be ready to face possible future mass disaster scenarios. Large or small, one can never be ready for all scenarios. The task of primary medicolegal death investigation falls in many jurisdictions on the coroner, who must be prepared for any death(s) falling under their jurisdiction. One of the worst-case situations involves mass shooting fatalities. These happen suddenly and without warning. The Las Vegas shooting is an example of such a potentially overwhelming investigation. Lessons learned from the experience provide insights in readiness if such awful acts occur again. Although fortunately involving fewer fatalities, smaller-scale investigations can be no less troubling and warrant similar media scrutiny. The racially motivated slaying of nine parishioners with clergy at a Bible study group shocked a city, a nation, and the world. How could this happen here? In a city not unfamiliar with shocking cases (nine firefighters dead in the line of duty, a suspect shot in the back while fleeing, and the like), these are another day at the office for a medicolegal death investigator.

When the deceased come in, the task of examination of the remains and telling their story falls upon the forensic pathologist. As with other forensics professionals, the stories are waiting to be told, awaiting only an attentive ear and an eloquent voice. Personal recollections of the medical examiner can make for best-selling non-fiction. But, are there lessons to be learned about and in the telling?

Eventually, the press interest in cases comes to center on the courtroom where evidence—good or bad—is presented to a presumably objective arbiter of fact. With intense pre-trial and in-trial scrutiny, including live televised trials, attorneys have had to adapt and perfect new strategies to best present their cases. Lessons learned from a career trial criminal defense attorney (ongoing) –turned-TV journalist offer a unique perspective on the good, the bad, and the ugly of courtroom as entertainment and the media as fact source.

Often downplayed in the entire media hoopla surrounding a case is the victim, without whom there would be no story. Every victim has a story, but not everyone has someone to tell it. In a unique circumstance, the surviving family can tell the decedent’s tale in such a way as to capture not only public but also professional interest. The session closes with the brother of a homicide victim, who shared his sister’s story in an award-winning documentary. The session will close with a special screening of her story: *Alice is still dead*.

In the “sweet science” of pugilism, participants are assessed according to their literal physical dimensions—the “tale of the tape.” In the modern era of ubiquitous video, the forensic sciences have been and continue to be judged based on a different, but no less real physical comparison: the taped record of events. Properly anticipated and thoughtfully pursued, information flow can be successfully managed and utilized, leaving both sides, the pursuit of truth and the quest for justice, as winners.

#### Media, Scrutiny, Journalism

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## BS1 The Path Forward Continues: What’s Happening in Forensic Standards

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**Learning Objective:** Participants will learn about updates in standards development in the United States and internationally from United States Standards Developing Organizations (SDOs). Participants will also get an update from the Organization of Scientific Area Committees (OSAC) for Forensic Science regarding how they are working with the SDOs and encouraging forensic science service providers to implement standards.

Participants will gain a better understanding of the progress of consensus standards development, including submission, drafting, public and committee commenting and resolutions, and finalization. Participants will also realize the importance of public input into the standards process and will learn how to participate, even if they are not part of an SDO consensus body or committee.

**Impact Statement:** The 2009 National Research Council (NRC) stressed the lack of standardization in forensics. The advent of OSAC, administered by the National Institute of Standards and Technology (NIST), and the AAFS Standards Board (ASB) has resulted in several years of concerted efforts to identify gaps in needed standards and begin their development using the voluntary consensus standards development process. All forensic professionals need to be aware of new and emerging standards in the various forensic disciplines, their importance, and how they can be implemented.

In 2009, the NRC published the Report, *Strengthening Forensic Science in the United States: A Path Forward*, which summarized the challenges facing the forensics community and the lack of mandatory standardization, certification, and accreditation. From this report, the Department of Justice (DOJ) and the NIST signed a Memorandum of Understanding that led to the establishment of the National Commission on Forensic Science (NCFS) and the NIST-administered OSAC for Forensic Science.

Presenters will provide an overview of the standards currently in development, those soon to be published, and the new standards published by various SDOs. New standards include those related to interdisciplinary topics, such as evidence collection and labeling, interpretation and reporting, and discipline-specific standards in the areas of criminalistics, toxicology, digital evidence, anthropology, 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.

Presenters will describe the process that a document goes through from inception through drafting, commenting, voting, and publication. This overview will give participants the education they need to participate in the standards development process.

OSAC has been working closely with various SDOs to bring the community together to define requirements for disciplines to submit standards through the development process. OSAC’s role in the standards development process and promotion of implementation of these standards will also be covered.

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### Standards, Consensus, Forensic Science

### BS2 Autopsy of a Crime Lab: The Evolving Approaches Toward Forensic Evidence in Court

*Brandon Garrett, JD\*, Duke University School of Law, Durham, NC*

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**Learning Overview:** This presentation will survey the current state of litigation and judicial review of admissibility of forensic evidence, as well as providing an overview of a recent book, *Autopsy of a Crime Lab*, that surveys the sources of error in forensic work and approaches designed to improve the process and safeguard reliability.

**Impact Statement:** This presentation will provide an overview of the book, but particularly focus on legal research and developments in the courts as judges have evolved in their approach toward forensic evidence. Studies examining how lay jurors evaluate forensic testimony in court will be summarized.

“That’s not my fingerprint, your honor,” said the defendant after Federal Bureau of Investigation (FBI) experts reported a “100-percent identification.” They were each wrong, and Brandon Mayfield was later cleared. How does error, even in high-profile cases, occur? Is it the quality of the latent fingerprint, the role of databases, individual examiner error, cognitive bias, or uncertainty inherent in a method that can lead to error? How should lawmakers, labs, and courts approach each of these challenges? The recently published book, *Autopsy of a Crime Lab*, is the first to catalog the sources of error behind a range of well-known forensic evidence, from fingerprints and firearms to forensic algorithms. At the crime scene, the book explores the need for scientific standards regarding evidence collection. Moving to the lab, the book explores quality controls, human factors, and the need for further studies to validate basic premises of widely accepted techniques. Turning to individual experts, the book asks how proficiency tests could be used to better assess individual performance, and the need for standards governing report writing and testimony. Reaching the courtroom, the book examines how concepts of judicial gatekeeping have slowly evolved, regarding discovery, testimony, and expert admissibility.

Taking us into the lives of the wrongfully convicted or nearly convicted, into crime labs rocked by scandal, and onto the front lines of promising reform efforts driven by professionals and researchers alike, *Autopsy of a Crime Lab* describes the forensic landscape and recent efforts to place evidence, from the crime scene to the laboratory to the courtroom, on a sound scientific footing.

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**Gatekeeping, Testimony, *Daubert***



### BS3 Procedures for Breaking the Zodiac Killer's Code

*Jeanne Anderson\*, Federal Bureau of Investigation Laboratory, Quantico, VA; David Oranchak\*, Roanoke, VA*

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**Learning Overview:** Attendees will learn about the forensic cryptanalysis of the Zodiac Killer's 50-year old unsolved cipher, how this code was broken, and the procedures used by the Federal Bureau of Investigation (FBI) Laboratory in cases such as this.

**Impact Statement:** This presentation impacts the forensic science community by bringing awareness of forensic codebreaking capabilities.

The field of forensic cryptanalysis, or codebreaking, is a little-known but well-established forensic discipline that, when applied, can produce reliable and informative results. Cryptanalysis can be applied to records of illicit businesses, such as drug distribution or manufacturing, human smuggling, and commercial sex, as well as coded messages.

The FBI Laboratory houses the Cryptanalysis and Racketeering Records Unit (CRRU) and is staffed with forensic cryptanalyst examiners trained to decipher coded messages and cryptic illicit business records. This forensic analysis relies on standard operating procedures based on steps developed in the early 1900s by a United States Army cryptologist and that remain accepted by forensic and academic cryptologic communities today. These procedures provide a framework for the analysis of codes and records that apply regardless of crime, context, or record type. Despite this framework's historical basis, it remains largely unknown by the forensic community; this presentation will inform attendees about this forensic capability. Further, this presentation will introduce one such codebreaking case, that of the never-caught Zodiac serial Killer. This case involved a coded message that remained unsolved for 50 years before it was broken this past December. This development provided the law enforcement community with new material on an unsolved serial killer case and the cryptologic community with a new encipherment technique that confounded professional and amateur cryptanalysts for 50 years.

One of the three-person team who broke this message will provide background on techniques employed for over a decade of work on the Zodiac Killer's code, including those techniques that eventually helped decrypt this coded message as well as how these techniques may apply to remaining unsolved coded messages. An FBI Cryptanalyst Forensic Examiner will present on law enforcement's approach to coded messages, specifically the standard operating procedures used for analysis of records and codes such as this. Several remaining unsolved coded messages will be introduced for those interested in applying any of the presented cryptanalytic techniques to actual unsolved codes.

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**Cryptanalysis, FBI Codebreaking, Zodiac Killer**

### **BS4 Pursuing Truth Through Forensic Evidence—But Does the Family Believe?**

*T.L. Williams, MFS\*, United States Army Criminal Investigation Command, Quantico, VA; Phillip Curran, MFS\*, United States Army Criminal Investigation Command, Quantico, VA; Tanya Marlow, MFS\*, United States Army Criminal Investigation Command, Quantico, VA*

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**WITHDRAWN**

### BS5 Helping the Marines Correct the Historical Record: Identifying the Iwo Jima Flag Raisers Using Photographic Comparison and Facial Recognition

*Richard Vorder Bruegge, PhD\*, Federal Bureau of Investigation, Quantico, VA*

**Learning Overview:** Attendees will learn how Digital and Multimedia Sciences techniques, including photographic comparison, facial recognition, and 3D face modeling, were used to help the United States Marine Corps correct the historical record in regard to the individuals depicted in the iconic “Iwo Jima Flag Raising” photograph by Joe Rosenthal. This case provides an example of how new techniques may be tested against unusual types of evidence in a matter of historical significance.

**Impact Statement:** The forensic science community will see an example of how established and innovative forensic science techniques can be “stress tested” in unusual circumstances to help further define the limits of these techniques.

On February 23, 1945, United States Marines raised an American flag on Mount Suribachi, Iwo Jima. A photograph depicting that event taken by Associated Press photographer Joseph Rosenthal immediately became iconic in Marine Corps and United States lore, with the image serving as the basis for the United States Marine Corps War Memorial in Washington, DC, and the National Museum of the Marine Corps in Quantico, VA. While most Marines will tell you that the individual names of those in the photograph do not matter, historians, and many in the public, want to be able to put names to the faces.

Previous Marine Corps reviews led to updates of the record in 1947 and 2016, but after the 2016 Huly Board findings were released, historians Dustin Spence, Stephen Foley, and Brent Westemeyer brought forth additional evidence indicating that further changes were needed. As a result, a third Board was convened in 2018. This “Bowers Board” requested the assistance of the Federal Bureau of Investigation (FBI) Digital Evidence Laboratory (DEL) in analyzing photographs and film footage from that day to help establish, once and for all, which Marines are depicted and in which positions around the flag.

The flag raised in the Rosenthal photograph was the second raised on the summit that day, replacing a smaller one. Several photographers, including one with a 16-mm movie camera, were also present and captured multiple photographs and film recordings of events on the summit that day, culminating in a series of celebratory photos known as the “Gung Ho!” photos. The combination of these photographs and film recordings make it possible to track the movements of various individuals during this sequence and establish their presence through image analysis (photographic comparison), primarily based on their faces and details of their helmets, clothing, and gear.

Through this examination process, the identities of four of the six individuals around the flagpole in the Rosenthal photo can be established with either “Strong Support” or “Extremely Strong Support,” while there is “Some Support” or “Limited Support” for the other two. Facial recognition technology and 3D-facial modeling software were also utilized in support of these analyses. When combined with further information from historical records, these findings offered a compelling case for the Bowers Board to declare in 2019 that the record is now complete.<sup>1</sup>

Requests like this—which do not involve criminal or civil litigation—allow multimedia evidence labs to not only stress test their existing standard procedures on different materials and content, but also allow them to experiment with new techniques and procedures under more “realistic” conditions than may otherwise be available. In this case, facial recognition technology and 3D-modeling software were utilized in support of traditional image analysis techniques for photographic comparison. Through this process, potential limitations of these technologies were identified. This presentation will provide attendees with a better understanding of the process involved in this image analysis examination, as well as how the facial recognition and 3D-modeling software were leveraged in this case, along with the potential limitations.

#### Reference(s):

- <sup>1</sup> *Investigating Iwo: The Flag Raisings in Myth, Memory, & Esprit de Corps*. Contributing editor: Breanne Robertson, PhD., Marine Corps History Division, Quantico, Virginia, 2019, LCCN 2019035917 | ISBN 9781732003071 (Also Available at: [https://www.usmcu.edu/Portals/218/Investigating%20Iwo\\_WEB2.pdf](https://www.usmcu.edu/Portals/218/Investigating%20Iwo_WEB2.pdf))

#### Facial Recognition, Image Analysis, Photographic Comparison

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## BS6 Electrical Torture, Electrocutation, and Homicide—The Istanbul Protocol

*Helmut Brosz, BASc\*, Forensic Sciences International Group, Markham, Ontario Canada; Renee Knight, BSFS\*, Forensic Sciences International Group, Markham, Ontario, Canada*

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**Learning Overview:** This presentation will provide an overview in identifying electrical torture injuries through a foundational background in body resistance to electricity and electrocution scenarios. After attending this presentation attendees will: (1) have an understanding of what is Electrical Shock Torture as defined by the Istanbul Protocol; (2) Mechanisms of Electrocutation; and, (3) the variables to be applied in the identification of electrical torture, injury, and homicide.

**Impact Statement:** This presentation will impact the forensic science community by improving the ability of Authorities Having Jurisdiction (AHJ), including investigators, medical examiners, lawyers, prosecutors, and engineers, to identify when an electrical torture or other electrical incident has taken place. Attendee knowledge of electrical shock injuries, torture, and homicide will be increased.

AHJ attending to a suspected electrical torture scene or homicide scene involving electrocution sometimes have difficulty discerning between natural death and death due to electricity. On average in North America, between 500 and 1,100 electrocutions occur annually, including suicide, auto-erotic, accidental, homicide, etc. The statistics for electrical torture are hard to come by; however, this form of torture “Parilla” appears to be practiced in certain South American and far Eastern Countries. There are also approximately ten times as many arc flash burn injuries and more than ten times as many non-fatal electric shocks. Some minor shock situations result in fall reactions that can lead to death and injury. These low-voltage shocks/electrocutions frequently leave no visible marks on the person. High-voltage electrocutions mostly leave distinctive marks and catastrophic injuries.

In scenarios involving electrical torture, readily identifiable marks may not always be available. Scenarios involving an individual being shocked in the presence of water may sometimes obscure contact entry and exit marks. Scene photos, hospital admission notes and sketches, autopsy photos, and reports are important data to be created, then assembled, studied, and analyzed as set out in the Istanbul Protocol.

The review and study of applicable standards and codes can assist the AHJ or investigator. For example, the Istanbul Protocol provides a definition on what is deemed as electrical shock torture, the device mechanisms utilized, and the various pathological outcomes of the torture relating to independent and dependent variables. For electrocution situations, the National Electrical Safety Code (NESC), Occupational Safety and Health Administration (OSHA) 26CFR1910, 26CFR1926, National Fire Protection Association (NFPA) 70E, and California General Order 95 may apply.

Variables in identifying electrical shock include voltage, body resistance, time, and the paths the current takes through the body. Through these factors, reconstruction and forensic analysis can be conducted in identifying and interpreting the method and potential motive for electrical torture through case presentations, examples, and artifacts.

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### Torture, Electrocutation, Istanbul Protocol

### BS7 Forensic Nursing Science: A New Section for a New Scientific Era

Nancy Cabelus, NDP\*, University of New Haven, New Haven, CT; Virginia Lynch, MSN\*, Forensic Nurse Consultants, International, Divide, CO; Joyce Williams, DNP\*, Stevenson University, Owings Mills, MD; Amy Carney, PhD\*, California State University, San Marcos, CA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the history of this dynamic discipline, the inception of forensic nursing worldwide, and how forensic nurses contribute to clinical practice, research, and policy.

**Impact Statement:** This presentation will impact the forensic science community by presenting exemplars of the application of care of victims, survivors, and perpetrators of violence of trauma.

In 1991, the American Academy Forensic Sciences (AAFS) distinguished forensic nursing as a scientific discipline.<sup>1</sup> This pronouncement launched a movement now recognized worldwide. The following year, the International Association of Forensic Nurses (IAFN) was founded.<sup>2</sup> In 1995, the American Nurses Association (ANA) Congress of Nursing Practice bestowed the official status of a nursing specialty upon the science of forensic nursing.<sup>3</sup> The forensic aspects of nursing have long existed. Nurses have historically cared for victims of violence—both the living and the dead. In the 17<sup>th</sup> century, nursing had not yet become a science and midwives filled this role.<sup>4</sup> Two centuries later, Florence Nightingale established the first aspects forensic nursing when caring for the war wounded and dead.

Forensic nursing is a dynamic discipline that recognizes human violence and trauma through a contemporary domain of scientific knowledge, health care, human rights, social justice, public health, and evidence-based practice. Broadly defined, forensic nursing science is nursing applied to the law, following the long-established exemplar of forensic medical science. Forensic nurses are health care providers qualified in the examination and evaluation of patients presenting with forensic biomarkers of crime-related trauma and the clinical investigation of questioned deaths. Global violence and problematic social and cultural situations reinforce the need to define the dynamics, processes, and guidelines for forensic nursing practice as it evolves and endures change.

Educational requirements to practice in forensic nursing vary. The nurse must have, at a minimum, an Associate's degree in nursing but a Bachelor's degree is preferred. After completing the degree, the nurse must successfully pass the board exam in the state in which they intend to practice. After practicing clinical nursing for a year or more, s/he may desire to specialize in forensic nursing as a Forensic Nurse Examiner (FNE) generalist or select a subspecialty of this discipline. Opportunities for role development include the Sexual Assault Forensic Examiner (SANE) course or pursuing the forensic aspects of training as a legal nurse consultant, accident investigator, mental health nurse practitioner, or nurse death investigator. Educational opportunities may be offered in risk management agencies, law firms, correctional settings, or domestic violence shelters. Regardless of the specialized role, forensic nurses work with victims of all ages who have suffered abuse, neglect, sexual assault, trauma, or any other crime-related injuries. Forensic nurses may practice in hospitals and community settings, typically interfacing with the legal system and treating victims, suspects, or perpetrators of crime.

Policy development in forensic nursing has evolved as the professional role grows and changes. Across the United States, individual Boards of Nursing have evaluated the forensic nursing response and the gaps that are filled when forensic nurses work with law enforcement, attorneys, and fellow medical professionals. As individual states evaluate their Nurse Practice Acts and the impact they have on forensic applications, forensic nurses are examining the effect that state, national, and federal rules and policies have on their interactions with both individuals and institutions. Forensic nurses also shape policy, bringing change through advocacy and research.

Graduate forensic nursing programs are available, often online, and require a number of clinical hours (typically 500) in addition to the didactic requirement of 30–40 credits. Forensic nurses with a graduate degree may opt to teach, conduct research, or in a clinical practice. Advanced practice forensic nurses may pursue a doctorate degree in forensic nursing research (PhD) or a doctorate with a clinical forensic focus, the Doctor of Nursing Practice (DNP).

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#### Response, Forensic Nurse, Historical

## **W1 Transfer and Persistence of Physical Evidence: Deciphering Implications**

*Brooke Kammrath, PhD\**, Henry C. Lee Institute of Forensic Science and University of New Haven, West Haven, CT; *Elaine Pagliaro, JD\**, Henry C. Lee Institute of Forensic Science and University of New Haven, West Haven, CT; *Peter De Forest DCrim\**, John Jay College of Criminal Justice, West Haven, CT; *David San Pietro PhD,\** University of New Haven, West Haven, CT; *Pauline Leary, PhD\**, Federal Resources, Stevensville, MD; *Henry Lee, PhD\**, Henry C. Lee Institute of Forensic Science and University of New Haven, West Haven, CT

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**WITHDRAWN**

## W2 The National Institute of Standards and Technology (NIST) Forensic DNA Activities: Foundations, Research, and Standards

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**Learning Overview:** This presentation will review activities at NIST involving forensic DNA foundational studies, research, and standards.

**Impact Statement:** Presentations in this workshop will impact the forensic science community by contributing to an understanding of NIST activities in advancing knowledge and practice of forensic DNA through foundation studies, focused research, and development of documentary standards.

NIST has been Congressionally-funded to: (1) perform forensic science research, (2) administer the Organization of Scientific Area Committees for Forensic Science (OSAC) to promulgate scientifically sound standards, and (3) conduct scientific foundation reviews of forensic methods and practices. The NIST Applied Genetics Group has conducted cutting-edge research in DNA sequencing and other areas of forensic DNA for the past three decades.<sup>1</sup> The OSAC Human Forensic Biology Subcommittee has completed ten standards working with the AAFS Standards Board DNA Consensus Body.<sup>2</sup> Another two dozen documentary standards and best practice recommendations are in development. A DNA mixture interpretation foundation review was completed in 2021 that identifies and documents what is empirically supported and describes where future efforts are needed.<sup>3</sup> Valuable research on Probabilistic Genotyping Software (PGS) systems has also been conducted in the past year. In conjunction with the National Institute of Justice (NIJ) Forensic Technology Center of Excellence, NIST began work in 2020 with a DNA Interpretation Human Factors Working Group. Process maps describing decisions made during forensic DNA measurement and interpretation have been prepared by OSAC and reviewed by the Scientific Working Group on DNA Analysis Methods (SWGDM). This workshop examines these various efforts and shows how they are interconnected and will benefit the forensic DNA community and its stakeholders in the criminal justice system.

Effective training and continuing education are crucial to keep up with evolving forensic DNA technologies and applications. Forensic laboratories invest in the continuing education of their staff. Universities with forensic science programs seek to prepare their students to be future contributing caseworkers. Stakeholders in the criminal justice system (e.g., law enforcement personnel, lawyers, and judges) using DNA results also benefit from regular training and continuing education to understand capabilities and limitations of methods and practices. Forensic scientists, students, and stakeholders can profit from drawing from a common informative knowledge base.

NIST forensic science efforts identify, consolidate, and share core principles, advance the state-of-the-science, and provide supporting publications and documentary standards to encourage deeper learning and understanding of forensic DNA. Based in part from the foundation review on DNA mixture interpretation and through discussions with experienced forensic DNA scientists, an initial list of 500 informative publications across 26 topic categories in forensic DNA was presented as an AAFS workshop in 2021.<sup>4</sup> An update on this list of most valuable publications will be provided as well as initiatives to provide additional training for practitioners and stakeholders.

### Reference(s):

1. NIST Applied Genetics Group. See <https://www.nist.gov/mml/biomolecular-measurement/applied-genetics>.
2. OSAC Human Forensic Biology Subcommittee. See <https://www.nist.gov/osac/human-forensic-biology-subcommittee>.
3. See <https://www.nist.gov/topics/forensic-science/interdisciplinary-topics/scientific-foundation-reviews>.
4. See [https://strbase.nist.gov/pub\\_pres/AAFS2021-W19-Handouts.pdf](https://strbase.nist.gov/pub_pres/AAFS2021-W19-Handouts.pdf).

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### DNA Mixture Interpretation, DNA Research, DNA Standards

## W3 Determining Sufficiency for the Identification of Gasoline

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**Learning Overview:** Attendees can expect an overview of the current gasoline identification limitations as well as an introduction to key diagnostic features of gasoline used in a statistical framework to enhance interpretation and identification.

**Impact Statement:** This workshop will detail chromatographic peak ratios with varied support for the identification of gasoline as well as a method to graphically demonstrate the overall statistical support for gasoline in the sample.

Currently, the consensus standard for identification and classification of ignitable liquids and ignitable liquid residues is ASTM E1618-19 Standard Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry. The guidance in this standard is limited to generalities about “the matching of the sample chromatogram with a reference ignitable liquid chromatogram obtained under similar conditions, noting points of correlation or similarities.” The document goes further to describe “Criteria for the Identification of Gasoline” that include brief pattern illustrations and hydrocarbon class characteristics. ASTM E1618 lacks definitive guidance for interpreting data when complex matrices are present that may alter or mimic patterns typified in gasoline. The standard does not have a mechanism to evaluate the strength of a possible agreement between the unknown sample and a reference ignitable liquid. The result is subjectivity potentially causing two analysts to reach different conclusions regarding the presence or absence of gasoline in a sample. The information to be presented fills those gaps with a method that is intuitive to explain and is relevant to fire debris analysts as an extension of what they are doing in the lab. By applying a statistical method based upon peak pair ratios, the level of subjectivity is greatly reduced and the strength of a potential association between the unknown sample and a reference ignitable liquid can be easily assessed.

The information to be presented provides instructions to build a sufficiency graph with decision lines to demonstrate the sufficiency of the data for the identification of gasoline to further strengthen the data interpretation process and provide transparent documentation. The objective of this information is to make the fire debris analysis process more standard, objective, and transparent by establishing a validated method with quantitative measures that include the implementation of documentation methodology and verification.

The identification of gasoline in a fire debris sample is dependent on the subjective interpretation of chromatographic peak patterns. As the complexity of the matrix contributions increases, the subjectivity of the identification also generally increases. Unfortunately, limited information has been published to assist in the formation of conclusions in the presence of complex matrix. These subjective interpretations can lead to interpretation inconsistencies among examiners and no objective method for the trier of fact to determine the basis for these conclusions.

In an effort to strengthen data interpretation, an objective, statistically based method for interpreting a sample to support a gasoline identification will be introduced.<sup>1</sup> This methodology directly translates to the visual pattern recognition techniques currently employed in the field and can be completed by a trained examiner in a short period of time. The developed method mirrors the theoretical sufficiency graph developed for use in friction ridge analysis, but augments this method by creating plot values that represent the Total Ion Chromatogram (TIC) and Extracted Ion Profile (EIP) features based upon peak ratios of key diagnostic features. Displayed on a sufficiency graph, these plot values visually demonstrate support for identification or non-identification of gasoline by placement on the graph with decision lines.

This intermediate workshop is designed for fire debris examiners and will use presentations and hands-on activities to: describe the current state and limitations of gasoline identification; present the project design and experimental results; direct the attendees in application of the process; demonstrate software (Excel®) techniques for calculating peak pair ratios and charting results; provide practical exercises for completing an analysis of a complex sample; and provide discussions on the validation and implementation of the technique in a laboratory setting. A special emphasis will be included on the chromatographic features showing high statistical support for gasoline.

By the completion of the workshop, the attendee should have the framework to evaluate the sufficiency of gasoline identification using a statistical method that enhances current visual pattern recognition techniques used in most forensic laboratories.

In order to get the most from the practical exercises of this workshop, each attendee (or group of 2–4 attendees) should have a laptop computer with Microsoft® Excel® and Agilent® ChemStation® or another Gas Chromatography/Mass Spectrometry (GC/MS) data processing software. Attendees may, but are not required to, bring hard copies and electronic data files from known gasoline samples acquired using existing laboratory methodologies.

### Reference(s):

- <sup>1</sup> Brenda Christy; Kelsey Winters; Alexandria Rossheim; Reta Newman; Larry Tang. A foundational study of fire debris interpretation using quantitative measures of chromatographic features in gasoline and the use of graphical display to demonstrate data sufficiency. *Forensic Chemistry*, Volume 24, 2021, 100337, ISSN 2468-1709, <https://doi.org/10.1016/j.forc.2021.100337>. (<https://www.sciencedirect.com/science/article/pii/S2468170921000333>).

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### Gasoline, Fire Debris, Interpretation



## W4 The Application of Evaluative Reporting for Forensic Handwriting Examinations

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**Learning Overview:** Attendees will learn how to apply Evaluative Reporting for evidence interpretation and conclusions. Handwriting comparisons will be used as examples. There will be a short lecture, followed by a demonstration using a mock case. The majority of the class will be hands-on group practice and feedback.

**Impact Statement:** This presentation will impact the forensic science community by describing modifications to the current interpretation method and reporting conventions that can help elucidate the evaluation process and advance forensic document examination as a science.

This workshop will focus on the application of the “Evaluative Approach” to evidence interpretation and reporting conclusions for handwriting examinations. Using this approach requires no change in the examination and comparison of handwriting but does involve adjustment in the evaluation if logical reasoning is to be followed. The fundamental principles of this method of forensic interpretation require that evidence be interpreted: (1) within a framework of relevant case circumstances, (2) by considering at least two competing propositions, and (3) by reporting the probability of the evidence given the propositions rather than the probability of the propositions themselves. Following these principles will allow the examiner to provide logically coherent and balanced evaluations.

Attendees will receive mock cases to examine, but not evaluate, prior to commencement of the workshop. The workshop will begin with a brief introduction of the key principles of the approach and general theory, followed by a demonstration of the method using one of the mock cases distributed earlier. For the remaining cases, attendees will break into groups and evaluate evidence using the approach. After each group breakout session, class will reconvene for discussion with the instructors.

The target audience of this workshop is forensic document examiners who are receptive to further awareness of the logic behind the evaluation process. A prior understanding or exposure to the approach is recommended but not necessary.

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### Evaluative Reporting, Evidence Interpretation, Conclusions

## W5 Unseen Threats

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**Learning Overview:** Attendees will gain a better understanding of public threats in respect to emerging drugs that are not often discussed (e.g., electronic cigarettes and their role in drug use, the effect of polydrug overdoses in toxicology and emergency rooms, online resources and how they can be used to predict future emerging drugs, fentanyl supervised consumption sites, and drug concealment techniques). Attendees will also gain awareness of innovative techniques used to address challenges in these areas (e.g., Direct Analysis in Real Time-Mass Spectrometry [DART®-MS] for the rapid screening of the chemicals in electronic cigarettes, and real time fatal overdose surveillance).

**Impact Statement:** Attendees will become aware that drug-related deaths continue to significantly impact communities. There are topics that influence the illicit drug landscape in these communities that are not frequently discussed.

The dynamic landscape of illicit drugs and its role in overdoses continue to be at the forefront of community efforts in maintaining public safety. However, there are topics that need more discussion to obtain better clarity on their influence on drug-related deaths. This workshop is geared to raise awareness of these emerging drug threats that deserve more attention and focus (e.g., electronic cigarettes and their use with drugs, the role of polydrug in overdoses and the challenges they present in emergency rooms, drug concealment techniques, online sites, fentanyl supervised consumption sites).

This workshop will discuss how electronic cigarettes are being adopted by recreational drug consumers for their culture acceptability and concealability. E-liquids contain chemicals considered safe for oral consumption, creating an aura of safety for consumers. While the E-cigarette, or Vaping, product use Associated Lung Injury (EVALI) epidemic exposed the toxic nature of vaping, the e-cig industry has facilitated the public consumption of recreational drugs. While traditional analytical techniques have been used to characterize the chemical composition of e-liquid formulations and their resulting aerosols, a more rapid screening technique is highly desired. This workshop will discuss the use of the DART®-MS system and method for coupling directly with commercial electronic cigarettes without sample preparation.

In addition, the complications of polydrug in toxicology testing and the challenges they present in death determination will be discussed. Interesting postmortem case studies, involving polydrug overdoses and instances where an unexpected analyte was detected after the initial information told a different story will be presented. This workshop will also explore the treatment of emergency department patients (with illicit drug overdose symptoms) and the possible unknown co-exposures that create challenges in treatment.

While mortality data, based on death certificates, are the traditional means of tracking the opioid/drug overdose crisis for law enforcement and public health, other innovative ways to obtain and evaluate data are being explored. This workshop will show how real time fatal overdose surveillance uses investigation, in-house drug and paraphernalia testing, rapid toxicology, and family interviews to detect emerging threats and more quickly certify overdose deaths. Another example is the use of online resources to provide insights into next-generation threats, especially those related to drug use and emerging synthetic drugs. Online web market sites detail in real time the newest drugs being sold, most of which are produced to evade national and international regulations. As drugs appear on these sites, discussions on drug use forums (e.g., Reddit) often follow and may be accompanied by anecdotal information about drug activity and adverse effects. Discussion will include how monitoring these data streams has proven useful for predicting the next wave of drug threats and better preparing forensic laboratories.

Another area often overlooked is the complex concealment techniques used by drug traffickers to circumvent law enforcement. With constantly changing drug trends, varying supply routes, and a wide variety of concealment methods, intercepting narcotics coming into the country is never a straightforward endeavor. An overview of some of the more unique concealment methods utilized by drug traffickers will be presented, as well as some of the sampling and extraction methods used to prepare these submissions for analyses.

In conclusion, the material presented in this workshop will be geared to bring more clarity to some of the unseen threats affecting drug-related deaths.

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### Vaping, Polydrug Overdoses, Predicting Drug Threats

## W6 Impairment: A Look at Causes, Data, and Policies

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**Learning Overview:** Attendees of this workshop will gain: (1) expertise in the role of Drug Recognition Experts (DREs) and their role in impairment investigations; (2) detailed knowledge of the role of fatigue in transportation accidents and an understanding of the factors impacting fatigue; (3) in-depth knowledge of the most recent recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities; (4) an appreciation of the roles of alcohol and cannabinoids in driving impairment and various strategies to control their use within the population; (5) an awareness of the tools available, roadside screening of oral fluid samples, and the policies regarding these samples; and (6) a detailed overview of the Academy Standards Board (ASB) Standards and the role they play in impairment investigations from testing in the laboratory all the way through to courtroom testimony.

**Impact Statement:** This workshop will impact the forensic science community by providing a detailed overview of recent policy changes and current developments within impairment testing.

From the time a subject is stopped by the police and assessed by a DRE until testimony at court, various policies, data, and circumstances come into play. This cross disciplinary workshop is equally relevant for forensic toxicologists, policy makers, pathologists, lawyers, and anyone who has a general interest in impairment.

One of the frequent requests of the forensic toxicologist is to assist the court with impairment determinations. A DRE is an individual who has successfully completed all phases of the Drug Evaluation and Classification Program's (DECP) training requirements for certification. A DRE is skilled in detecting and identifying persons under the influence of drugs and in identifying the category or categories of drugs causing the impairment.

Fatigue-related impairment can result from sleep loss, time of day, time on task, medical issues, drug use, or a combination of those factors. It can affect cognitive performance in many ways, most notably in a loss of sustained vigilance, which can lead to errors and—in some cases—major safety events.

The Driving Under the Influence of Drugs (DUID) recommendations were based on a survey sent to laboratories throughout the United States and Canada about testing practices, scope of testing and cutoffs, matrices tested, and compliance with the 2017 recommendations. Tier I and Tier II scope and cutoffs for screening and confirmation were re-evaluated and updated based on analysis of the survey results, ongoing research, current levels of testing, and concentrations in DUID cases.

There is a lack of linear cannabis dose concentration effect and therefore it can present challenges for impairment interpretation. The pharmacokinetics of  $\Delta 9$ -Tetrahydrocannabinol (THC) after smoked, vaporized, and oral cannabis will be presented, including the lack of a linear concentration-effect curve and future drug policies that may lessen the impact of cannabis medicalization and legalization.

Alcohol and cannabis are two of the most frequently detected drugs in serious/fatal injured drivers. Studies of the relative risk of a serious injury or death from impairment for these two substances range from slightly to extremely increased risk. Studies by the National Highway Traffic Safety Administration indicate that about 4–6 percent of road users were positive for both alcohol and cannabis in 2020. The combined effect of alcohol and THC on driving impairment has been studied and found to be greater than either drug effect alone.

Oral fluid has continued to gain popularity as an acceptable matrix for drug detection in impaired driving cases and is an approved specimen in 22 states. There are now available tools designed for screening oral fluid samples at the roadside, considerations for laboratory testing and the implementation of oral fluid screening in DRE certifications must be put into place.

Polydrug use in DUID cases at different blood alcohol concentrations is often not included in toxicology casework as the focus is placed on alcohol testing. Other drug trends and a comparison between blood and oral fluid drug concentrations in DUID cases can be important demographic data to obtain. The impact of stop testing limit programs will be evaluated by comparing the advantages and disadvantages of different DUID testing schemes and workflows.

Forensic toxicology standards published by the Academy Standards Board (ASB) through the American National Standards Institute (ANSI) have led the way to promoting consistency in common practices within the field. ANSI/ASB Standard 120—*Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigation* and ANSI/ASB Standard Best Practices Recommendation 037—*Guidelines for Opinions and Testimony in Forensic Toxicology* are critical for improving the testing performed in DUID investigations and how analytical results and expert opinions are presented through courtroom testimony.

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### Impairment, Standards, Policy

## W7 Histology for Non-Pathologists

*Katherine Maloney, MD\**, Erie County Medical Examiner Office, Buffalo, NY; *Milad Webb, MD, PhD\**, Hillsborough County Medical Examiner Office, Tampa, FL; *Ashley Perkins, DO\**, Hillsborough County Medical Examiner Office, Tampa, FL; *Omar Rayes, MD\**, Wayne County Medical Examiner Office, Detroit, MI; *Teresa Nguyen, MD\**, Wayne County Medical Examiner Office, Detroit, MI; *Kelly Devers, MD\**, Hillsborough County Medical Examiner Office, Tampa, FL

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**Learning Overview:** Attendees of this workshop will learn the basics of histology as well as receive an introduction to the histologic interpretation of infection and trauma, including trauma to the brain and spinal cord.

**Impact Statement:** This presentation will impact the forensic science community by providing non-pathologists with an understanding of the process of histology interpretation and, in particular, its relevance to their forensic specialty.

During their anatomic pathology training, forensic pathologists learn the art of histopathology, which involves looking at small sections of tissues under the microscope for identification and interpretation. The results of this examination can be used to determine the cause and manner of death, extent and type of disease process, and nature of identified abnormalities. Although extensive training is provided to pathologists on this topic, little to none is given to other forensic practitioners, even those whose work can be affected by the results of these examinations. It is not uncommon for histology findings to be referenced in a court of law in both criminal and civil proceedings. Additionally, while the interpretation of certain findings is nearly universally agreed upon by well-trained pathologists, others can be fraught with controversy. The goal of this training is to provide non-pathologists with an introduction to histopathology such that they have an understanding of some of the basic concepts, terminology, and findings.

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### Histopathology, Infection, Trauma

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## W8 Subaerial Weathering of Bone

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**Learning Overview:** Attendees of this workshop will learn about the taphonomic processes causing subaerial weathering of bone and how to score weathering stages following the standard 0-5 scale from Behrensmeyer.<sup>1</sup> Participants also will learn about estimation of the Postmortem Interval (PMI) from the weathering stage and the other types of taphonomic alterations that commonly co-occur with surface exposure and how to tell the effects of subaerial weathering (cracking, delamination, and bleaching) from other causes that may produce similar effects.

**Impact Statement:** This workshop will impact forensic practice by teaching standard scoring procedures for subaerially weathered bone and the procedures for estimating PMI based on those scores. This workshop will encourage participants to gather additional taphonomic data to improve greater understanding of subaerial weathering and its regional variation.

Taphonomy is the study of an organism's changes from its death until the recovery of its remains. Multiple environmental processes act upon biological remains, including directly upon the bone itself. These processes vary by depositional environment and may alter bones in ways that are unique to remains from that environment. Investigators often recover remains from exposed terrestrial environments, and the bones often present a suite of distinctive characteristics, including carnivore gnawing, rodent wet- or dry-bone gnawing, algae formation, soil staining, and subaerial weathering. Recording of these taphonomic alterations is aided by standardization of methods. One of the earliest standardized analytical scales still in use in forensic anthropology and related disciplines is Behrensmeyer's stage 0 (unweathered) through 5 (severely weathered/falling apart) scale for bone weathering.<sup>1</sup> Bones undergoing subaerial weathering typically sun-bleach, dry, crack, and flake due to exposure to solar radiation, temperature fluctuations, freeze-thaw, wetting-drying, and mineral recrystallization. The end result is a fragmented bone that has become part of the lithosphere, completing its taphonomic cycle.

Significantly, subaerial weathering stages can be used to help estimate the PMI in the "middle" range between the time when decomposition and entomological studies leave off (typically less than one year) and the time at which radiocarbon dating becomes appropriate. Forensic anthropologists have few viable options within this interval and often must rely upon artifactual evidence or development of annual rings in tree roots found in direct association with remains. Subaerial weathering analysis also has the potential for misuse when temporal standards developed in different environments and species are applied uncritically to human remains to estimate the PMI, and these methodological difficulties are examined in this workshop.

Taphonomic alteration due to subaerial weathering should be distinguished from other sources, including other environments that bleach and/or crack bone, in order to understand the bone's recovery context. This patterning includes taphonomic alterations that may link a bone to a certain depositional setting, such as weathered remains from a surface deposit, even when the bone later gets moved to a new environment. Similarly, the movement of a bone from its original orientation while remaining in the same general location is often discernible from its subaerial weathering pattern. Since taphonomic changes vary by environment, regional differences are expected among these types of environments/sources of bone, and workshop participants are encouraged to gather and disseminate taphonomic data from their own areas so that regional standards can be developed in the future.

### Reference(s):

<sup>1</sup> Behrensmeyer, A.K. (1978) Taphonomic and ecologic information from bone weathering. *Palaeobiology* 4(2):150-162.

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### Forensic Taphonomy, Subaerial Weathering, Postmortem Interval (PMI)

### W9 Forensic Science Standards Development and Implementation . . . You Want Me to Do What?

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**Learning Overview:** The goal of this workshop is to discuss the development process of forensic science standards, how these standards are implemented in crime laboratories, and how they are viewed by the legal profession.

**Impact Statement:** This presentation will impact the forensic science community by enabling attendees to understand: (1) the process through which standards are developed and published; (2) issues with implementation of standards in crime laboratories; and (3) how the legal profession views and assesses the role of standards in court proceedings.

In 2015, the Academy Standards Board (ASB) was formed by the American Academy of Forensic Sciences (AAFS) to address concerns about lack of standardization in the forensic sciences. Erroneous convictions cast doubt on the scientific merit of some disciplines. It was thought that standardization would ameliorate these concerns by providing practitioners, attorneys, and the courts with documents procedures and best practices.

ASB is an American National Standards Institute (ANSI) -accredited Standards Development Organization (SDO), and it must meet ANSI's requirements before any document can be published. These requirements can be time-consuming, and many practitioners have become frustrated by the length of time that standard development takes. There have also been concerns about the lack of depth and content in some of the published standards. This workshop will explain the process through which an ASB document is published, and the reasons why the process cannot be rushed. Information on commenting on draft documents will be provided so that interested parties can give their input while the standard is in the development process. This is a very important part of the process that can take time, but is vital in publishing a sound document that meets the consensus of the stakeholders.

Once the ASB documents are published, crime laboratories may wish to adopt them for their manuals and reference documents. Federal, state, and local laboratories may each have different issues with implementation of the standards. Presentations will be given by practitioners and a laboratory director on their experiences in implementing standards in their laboratories. Forensic science does not exist in a vacuum, and the use of standards in other professions and how forensic science can learn from them will be discussed.

Finally, the value of the standards will, in part, be determined by the legal system. Standards may support the bases for opinions, and the courts will determine the weight of this evidence. Courts may pay close attention to varying conclusions given by experts who use the same standards, and experts who used different standards, or none.

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### Standards, Forensic, Implementation

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### W10 Inference From Evidence in Forensic Science and Pathology: Turning Something That Has Been Wrong Into Something Right

*Thomas Young, MD\**, Heartland Forensic Pathology, LLC, Kansas City, MO; *Ljubisa Dragovic, MD\**, Oakland County Medical Examiner's Office, Pontiac, MI

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**Learning Overview:** After attending this workshop, attendees will have learned which forms of inference—the reasoning involved in drawing conclusions from evidence—are highly reliable for truth and which forms are highly unreliable. Attendees will also learn how to apply proper inference to their forensic casework.

**Impact Statement:** This workshop will impact the forensic science community by revealing how demonstrably incorrect ways of inferring from evidence have led to numerous injustices from false accusations and incarcerations. This workshop will also disclose a reliable way to infer that is both scientifically and logically valid.

For several decades, many have criticized the forensic sciences, even to the point where one may wonder if what is purported as courtroom science is really “scientific.” Ever since DNA testing has repeatedly falsified the sworn statements and opinions of forensic doctors and scientists, the Innocence Project has leveled criticism at them, citing 52% of wrongful convictions to be from “misapplied forensic science” on their website, only second to the 63% they cite from “eyewitness misidentification.” In recent months and years, the criticism of forensic pathologists has also ramped up due not only to highly publicized cases but also to research purporting to demonstrate substantial cognitive bias in the ways forensic pathologists draw conclusions. Even seasoned forensic pathologists recognize the subjectivity of their conclusions. Is there a way for forensic scientists and pathologists truthfully to offer opinions from the witness stand made “to a reasonable degree of medical/scientific certainty”? Is there a way to put the “science” back into forensic science and pathology? Is there a way for forensic pathologists and scientists to offer opinions that are reliably truthful and helpful to triers of fact in a courtroom?

There is. The Inferential Test (IT) is a theorem or tautology of deductive logic applied to the forensic arena that is demonstrably always true, all the time, under all circumstances. The IT—“One can be reasonably certain if witness accounts of the past are consistent or not consistent with physical evidence in the present, but one cannot reliably surmise past events from physical evidence unless there is only one plausible explanation for that evidence”—points out what works and what doesn't work for drawing conclusions from evidence. This workshop will demonstrate the basis for the IT in both deductive (what can be guaranteed as truthful) and inductive (what can be asserted as probable) logic. It will also instruct attendees how to apply the IT properly in forensic casework, using examples from the media and from the case files of both instructors.

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#### Inference, Evidence, Forensic Science and Pathology

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### W11 Successful Strategies for the Accreditation of Crime Scene Units

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**Learning Overview:** After attending this workshop, attendees will have learned about the planning process for the accreditation of crime scene processes.

**Impact Statement:** In 2009, the National Academies of Sciences (NAS) issues their landmark Report, Strengthening Forensic Science in the United States: A Path Forward.<sup>1</sup> The Report recommended the accreditation of forensic laboratories and rightfully identified crime scene investigation as part of the forensic science community. However, since the publication of the Report, the accreditation of crime scene units globally has not kept pace with other disciplines. This presentation aims to reduce the perceived barriers that may stop an agency from pursuing accreditation of crime scene investigation processes, resulting in an increasing number of accreditations in this area, and thus improving the quality of forensic science at a systemic level.

It has been over a decade since the NAS issued their landmark Report on how to strengthen forensic science in the United States which, among other recommendations, advocated for the international accreditation of forensic services. Since the issuance of the report, the number of domestic and international forensic laboratories accredited under the applicable standards issued by the International Organization for Standardization (ISO) has increased dramatically. However, while most people agree that quality begins at the crime scene, the accreditation of crime scene activities has significantly lagged behind other forensic disciplines.

This workshop aims to address the misconceptions that may be stopping agencies from pursuing accreditation in crime scene processing. This workshop will share experiences in successfully supporting the accreditation of crime scene units both in the United States and internationally. A useful framework will be offered to manage the accreditation process and guidance on building a quality management system. Specific suggestions will be offered of how to address the ISO accreditation standards commonly viewed as barriers to accreditation of crime scene units such as measurement of uncertainty, proficiency testing, and validation. The workshop content will not be specific to any one accrediting body but will cover the general requirements under the ISO standards as well as the supplemental guidelines issued by the International Laboratory Accreditation Cooperative (ILAC) G19:2014—Modules in a Forensic Science Process.

Last, the workshop will cover the role of management in an effective quality system to include communication with stakeholders, management reviews, and dealing with non-conformities as they arise. Effective management is essential to extracting value out of accreditation. Accreditation requires not only an investment in time and money, but the desire for change, including modifications in work processes and organizational culture. The workshop will address resistance to change that may also inhibit the accreditation process by providing examples of how resistance has presented itself in past situations and suggestions on how it can be mitigated.

**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>.

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### Accreditation, Quality, Crime Scene



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## W12 Forensic Postmortem Radiology: Crossing the Border Between Radiology and Pathology

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**Learning Overview:** After attending this workshop, attendees will: (1) have learned the basics of how to get started in the field of forensic radiology; (2) understand the current state of the art and levels of evidence in forensic radiology; (3) have learned about applications to assist forensic pathologists and practitioners in medical death investigation; and (4) will be aware of potential resources available to interested practitioners.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with expertise from around the world on the use of best practices and the evidence base of forensic postmortem radiology.

It has been nearly 12 years since the publication of the National Academy of Sciences Report calling for the strengthening of the forensic sciences. In this seminal report, they stated the critical role that imaging plays in the documentation of findings sufficient for courts, for providing the opportunity for review by outside experts, as well as for allowing for reevaluation of evidence as medical knowledge advances. Since then, forensic radiology and imaging has grown significantly as a field around the world.

The “Virtual Autopsy” or “Virtopsy” utilizes Multi-Slice Computed Tomography (MSCT) or Postmortem Computed Tomography (PMCT) and Magnetic Resonance Imaging (MRI) combined with 3D imaging technology to create vivid images of the interior of the human body. CT scanning and MRI has been shown to present a better visual picture of some injuries, reduce the number of autopsies conducted to rule out occult injury, and document the extent of injury in accidents in greater detail. The advantages of the Virtopsy are that it is not invasive or destructive to tissues and can provide clear pictures of skeletal and soft tissue injury. It also provides some information when there is a religious objection to autopsy. Additionally, Virtopsy has the potential to detect internal bleeding, bullet paths, bone and bullet fragmentation, fracture patterns, brain contusion, and gas embolism, as well as occult fractures that are technically difficult to demonstrate during the traditional autopsy.

In the United States, only a few medical examiners/coroner’s offices (approximately 20) have access to this advanced medical imaging at this time and very few have the budget to purchase the expensive equipment or to build a suitable facility with staff to maintain it. The current public health crisis of opioid deaths is stretching an already strained medical examiner’s system with regard to autopsy caps set by the National Association of Medical Examiners (NAME) standards. Postmortem medical imaging can play a critical role in easing these demands for full autopsy on offices through triage screening from PMCT.

Within the past years, the National Institute of Justice (NIJ) has fully supported forensic imaging as a supplement to the traditional autopsy and as a triage and long-term evidence documentation tool. NIJ efforts have attempted to bring field standards from around the world and establish major research areas of need in order to help facilitate the field’s growth in the United States since it lags behind other countries in the field. Additionally, the post AAFS 2018 attendee survey indicated in several places the desire of members for more information on assisting forensic pathology workloads with the use of CT scans/documentation of trauma via imaging, even going as far as suggesting modifying of National Association of Medical Examiners guidelines to accomplish this. With the field well established in practice outside the United States, lessons learned from experts will help those interested in incorporating these technologies into their facility’s everyday practice. Exposure to emerging technologies in the field will also help practitioners be exposed to potential applications that will alleviate their workflow in an already overtaxed death investigation system.

This workshop will show how to start a program, give an overview of the current state of forensic imaging in the world and how future technologies may impact the field of forensics. A similar workshop was previously presented at an American Academy of Forensic Sciences (AAFS) conference by the same team and it was highly attended by members from different sections.

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### Radiology, Medical Imaging, Postmortem

## **W13 Forensic Techniques Applied in Military Investigations**

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**WITHDRAWN**

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## W14 Interdisciplinary Approaches to Age Estimation

*Sarah Ellingham, PhD\*, ICRC, Geneva, CH, Switzerland; Sara Zapico, PhD\*, New Jersey Institute of Technology, Newark, NJ, Joe Adserias Garriga, PhD\*, Mercyhurst University, Erie, PA; Douglas Ubelaker, PhD\*, Smithsonian Institute, Washington, DC; Sara Getz, PhD\*, University of Wisconsin-Plattenville, Plattenville, WI; Sakher AlQathani, PhD\*, King Saud University, Riyadh, SA, Saudi Arabia; Christian Crowder, PhD\*, Dallas County Medical Examiner's Office, Dallas, TX; Victoria Dominguez, PhD,\* City University of New York, New York, NY*

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**Learning Overview:** After attending this workshop, attendees will have a heightened understanding of: (1) age-at-death assessments as well as age assessment of the living from the anthropological and odontological point-of-view; (2) application of alternative techniques, based on the biochemical fundamentals of aging, to improving age estimates; and (3) age assessment implications in humanitarian contexts.

**Impact Statement:** This workshop will impact the forensic science community through the presentation of interdisciplinary approaches toward improving age assessments, encouraging collaboration and innovation among different forensic specialties. This workshop will further highlight the importance of age estimation in various contexts, including humanitarian applications.

Age-at-death estimation represents one of the key components for the creation of a biological profile to facilitate the identification of human remains. At the same time, it is one of the most challenging tasks, particularly in adult individuals where estimated age intervals tend to be very wide and point estimates are often biased. Additionally, in mass fatality events and other forensic contexts such as armed conflict, the presence of fragmentary remains can complicate the correct age assessment, and as a result, the identification of the individual.

This workshop will give an overview of the importance of age estimation in forensic science as well as other scenarios such as the context of migration; different age assessment methodologies from the point-of-view of forensic anthropology and forensic odontology in both juvenile and adult remains—living and dead—will be discussed; current state-of-the-art techniques based on the biochemical basics of aging will be introduced; finally the significance of age assessments in humanitarian contexts will be pointed out, drawing reference to implications of children not being issued a birth certificate, the 1989 United Nations Convention on the Rights of the Child and modern Asylum Law. The last session of the workshop will be devoted to practical examples of these aforementioned methodologies.

This workshop will demonstrate how the awareness of the problems faced with regard to age assessment, out-of-the-box thinking, the application of new and interdisciplinary techniques, and promoting collaborations among different forensic specialties such as anthropology, odontology, criminalistics are essential to improve age estimates, and as a result, the identification of the victim. Thus, this workshop will provide a step toward finding solutions to these challenges by improving the knowledge on the most state-of-the-art techniques available to forensic scientists.

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### Age Estimation, Forensic Anthropology, Forensic Odontology

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### W15 Unlocking the Truth: The Impact of DNA Testing and Legal Strategies in Post-Conviction Cases

*Lisa Mertz, MS\**, Office of Chief Medical Examiner, New York, NY; *Krista Currie, MS\**, Laurentian University, Sudbury, Ontario, Canada; *Andrew Schweighardt, PhD\**, Office of Chief Medical Examiner, New York, NY; *David McCallum, South Mineola, NY*; *Ken Klonsky, MA\**, Innocence International, Vancouver, British Columbia, Canada; *Ray Klonsky\**, Brooklyn, NY; *Oscar Michelen, JD\**, New York, NY; *Janis Puracal, JD\**, Forensic Justice Project, Portland, OR; *Jeff Reising, JD\**, Yolo County, Woodland, CA; *Hillary Blout, JD\**, For The People, Oakland, CA

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**Learning Overview:** Over the past 30 years, advancements in the field of forensic science have greatly impacted the criminal justice system. Advances in DNA testing and the development of new technology have led to an increase in exonerations of those wrongfully convicted.

**Impact Statement:** Attendees, armed with the knowledge of how best to efficiently and cost effectively process post-conviction cases, will be able to watch clips from a documentary to further inspire them to perform post-conviction DNA testing in their laboratories and form integrity units in their district attorney offices. This workshop will motivate those attending to realize that we can all participate in the quest for justice and, in the words of Dr. Rubin Carter, “. . . to live in a world where truth matters and justice, however late, really happens, that world would be heaven for us all.”

In this workshop, the case of David McCallum will be examined. David McCallum was convicted of murder when he was only 16 years old and served almost 30 years in prison for a crime he did not commit. His story was showcased by filmmaker Ray Klonsky in the documentary *Fight for Justice: David and Me*, released in 2014. This documentary explores the bond the two formed as they desperately tried to free David from jail. Attendees of this workshop will be presented with clips of the documentary and will hear both David and Ray detail their experiences.

This workshop will also highlight the legal struggles that David faced in his journey for freedom, which will be presented by his lawyer, Oscar Michelen, as well as others in the legal field who will talk about the legal hurdles involved in post-conviction cases. Also discussed will be the formation of conviction integrity units within the prosecutorial office. These units are instrumental to prevent and identify false convictions. In addition, attendees will hear from the father of Ray Klonsky, Ken Klonsky, who is the director of Innocence International, an organization founded by the late Dr. Rubin “Hurricane” Carter, a famed boxer who was also wrongfully convicted. Dr. Carter started Innocence International because of his own struggle. He had been wrongfully accused, convicted, and imprisoned for almost 20 years.

In discussing the DNA testing involved in David McCallum’s case, this workshop will present the challenges faced processing DNA evidence in post-conviction cases. Attendees will hear suggestions about how best to handle issues faced with vetting and processing older cases. This workshop will demonstrate how to use the efforts of multi-agency investigations and new technologies to efficiently and cost effectively perform DNA testing and aid in the exoneration of those wrongly convicted. Also discussed will be recent advancements in forensic science that have enabled us to revisit old, degraded, or compromised samples to determine if current DNA testing methods can now achieve a DNA profile. Today, techniques are so sensitive that one may obtain a DNA profile from just a few cells worth of DNA. With greater sensitivity, a whole new realm of possible evidence left behind at a crime scene can now be considered for DNA testing and help those who are wrongfully convicted.

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#### DNA, Post-Conviction, Justice

## W16 The Neurobiology and Toxicology of Aggression

*Alan R. Felthous, MD\**, Saint Louis School of Medicine, St. Louis, MO; *Marilyn A. Huestis, PhD\**, Huestis & Smith Toxicology, LLC, Severna Park, MD; *Scott Akridge, MD\**, Saint Louis School of Medicine, St. Louis, MO; *Joe Bou Nassif, MD\**, Saint Louis University School of Medicine, St. Louis, MO; *Felice F. Carabellese, MD\**, University of Bari, Bari, Italy; *Dean M. DeCrisce, MD\**, Coast to Coast Forensic Associates, Division of Behavioral Health Sciences, Elizabeth, NJ; *Alexandre Demais, MD\**, Institut Universitaire en Santé Mentale de Montréal, Montreal, Quebec, Canada; *Marissa Hirsch, MD\**, Saint Louis School of Medicine, St. Louis, MO; *Rebecca J. Houston, PhD\**, Department of Psychology, Rochester, NY; *Daniel S. Isenschmid, PhD\**, NMS Labs, Horsham, PA; *Graham R. Jones, PhD\**, Edmonton, Alberta, Canada; *Dayong Lee, PhD\**, Houston Forensic Science Center, Houston, TX; *Barry K. Logan, PhD\**, NMS Labs, Horsham, PA; *Katherine McArthur, MD\**, Saint Louis School of Medicine, St. Louis, MO; *Michael L. Smith, PhD\**, Huestis & Smith Toxicology, LLC, Severna Park, MD

**Learning Overview:** This workshop examines the nature and origin of aggression and the modulating effect of various drugs.

**Impact Statement:** This presentation will demonstrate how impulsive and premeditated aggression in humans is understood from neurocircuitry and neuroimaging studies, Central Nervous System (CNS) neurotransmitter involvement, and electrophysiology, including the P3, an Event-Related Potential (ERP) component elicited in the process of decision making that varies from person to person.

Aggression is described, categorized, and explained beginning with the neurocircuitry of defensive and predatory aggression in animal models. Drugs, recreational as well as therapeutic, can promote or reduce aggression. The chemistry, toxicology, and neurophysiology of cocaine, phencyclidine, cannabinoids, methamphetamine and alcohol, anti-impulsive aggression therapeutics and pharmacotherapies with paradoxical aggression will be discussed as potential modulators of aggressive behavior. The role of cocaine in violent deaths is examined, including a discussion of analytical results in violent deaths compared to other cocaine-related fatalities and other factors such as use of tasers and restraints. One well-documented PCP effect is aggression with post-surgical psychosis listed as a reason for its discontinuation as a marketed anesthetic.

A significant association was observed between Cannabis Use Disorder (CUD), difficulty managing anger, aggressive impulses/urges, and problems controlling violence in the past 30 days in a large sample of Iraq/Afghanistan-era veterans with Post-Traumatic Stress Disorder (PTSD), even accounting for demographic variables, comorbid symptoms of depression and PTSD, and co-morbid alcohol and substance use disorders. In addition, emerging research describes the long-term effects of cannabis on aggression in individuals with schizophrenia. Methamphetamine is a sympathomimetic stimulant whose use is associated with excited behavior, including agitation, suspicion, paranoia, impulsivity, and at higher doses and in patterns of more intense use, dissociation and delusions, sometimes leading to homicides and suicides. Novel synthetic psychotomimetics, including cathinones and synthetic cannabinoids, can induce aggressive behavior together with other mental symptoms, including delirium and psychosis. The unpredictable nature of alcohol-mediated aggression will be highlighted by the case of a young man who murdered a man he didn't know, in a local park, in front of witnesses, and later professed to have no recollection of events and suffered a total alcoholic blackout. Five Food and Drug Administration (FDA) -approved therapeutic agents for the treatment of other medical and mental disorders reduced the intensity and frequency of episodes of impulsive aggression or intermittent explosive disorder; however, on rare occasions, intake of these drugs lead to unusual aggressive responses. Mechanisms of action are unclear and the literature is limited; however, case studies showed anti-anxiety drugs that usually reduce aggressive behavior, such as benzodiazepines and selective serotonin reuptake inhibitors, may cause paradoxical aggressive reactions. Additionally, aggression may be an unintended side-effect of antiepileptics, anti-smoking medication, and anabolic-androgenic steroids.

### Neurobiology, Toxicology of Aggression, Drugs

## W17 Forensic Examination and Evaluation of the Fine and Subtle Elements of Signatures and Handwriting

Linton Mohammed, PhD\*, Forensic Science Consultants, Inc, Poway, CA; Lloyd Cunningham\*, Alamo, CA; Brett Bishop, BS\*, Washington State Police Crime Laboratory, Spokane, WA; Karen Nobles, MSFS\*, Forensic Document Services, LLC, Pensacola, FL

**Learning Overview:** This workshop will increase Forensic Document Examiners' (FDEs') knowledge regarding the fine and subtle elements of signatures.

**Impact Statement:** This workshop will impact forensic document examiners by increasing the accuracy and reliability of their opinions in signature and handwriting examinations.

The examination of signatures to determine whether they are genuine or simulated is a common task for FDEs who have demonstrated proficiency in this type of examination. In 2001, a study reported that FDEs had a Type I (calling a simulation genuine) error rate of 0.49% and a Type II (calling a genuine signature a simulation) error rate of 7.05%.<sup>1</sup> Another study found that FDEs had an error rate of 3.4% in their opinions regarding whether signatures were genuine or simulated.<sup>2</sup> Similar results were found for examinations conducted with photocopies and online documents.<sup>3,4</sup>

An auto-simulation (intentional distortion) is a strategy in which the writer attempts to disguise his/her signature with the intent to claim later that it was simulated. Auto-simulations often share similar characteristics with simulations. Studies cautioned that FDEs will find making a determination as to whether a signature is auto-simulated or simulated to be challenging.<sup>5-8</sup>

A five-year study was conducted in which 29, 811 opinions were expressed by FDEs on trials comprised of randomized questioned signatures that were categorized as genuine, auto-simulated (disguised), and simulated. The researchers found that, as a group, FDEs were significantly more confident at identifying writers' genuine signatures than identifying writers' disguised signatures or eliminating specimen writers from having authored simulated signatures. In this study, a 37.5% misleading score for disguised signatures as opposed to 4.8% for simulated signatures were reported.<sup>9</sup>

A recent study on Bias and Reliability of expert FDEs reported that the examiners were not biased by the agency of the submitter, or the instructions given, but were unreliable in the blind examination to determine whether the questioned signature was genuine, an auto-simulation, or a simulation. This study cautions that due to limitations of the study no definitive conclusions can be drawn regarding the applicability of the study's results to actual casework.<sup>10</sup> Nevertheless, the lack of reliability of many FDEs to differentiate between auto-simulated signatures and simulated signatures warrants concern, and insight is required as to the root cause of the problem.

A consideration of this workshop is that the observation and correct interpretation of the fine and subtle (inconspicuous) elements of signatures are required for the FDEs to come to reliable conclusions. The elements are caused by the unconscious writing habit of an individual who may not even be aware of these features.

This workshop will begin with a lecture session reviewing the research data regarding genuine, disguised (auto-simulated), and simulated signatures. Attendees will then be given practical problems to the hands-on session. They will be asked to present their results (in groups) and give reasons for their opinions. The workshop will then go into the fine and subtle details of the signatures in the problems to illustrate and explain how these details can be observed and evaluated.

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### Signature Examination, Handwriting Examination, Evaluation

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### **W18 An Advanced Bloodstain Pattern Analysis Hands-On Workshop With an Accompanying Principles of Bloodstain Patterns Lecture**

*Lawrence Renner, MA\*, Santa Fe Police Department (Retired), Santa Fe, NM*

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**Learning Overview:** After attending this workshop, attendees will have an increased understanding of bloodstain patterns, their relevance to crime scene reconstruction, the determination of events that occurred, and potential charges that could be issued.

**Impact Statement:** This workshop and the accompanying lecture will expand the understanding of lab and scene personnel who have not had previous bloodstain pattern analysis classes.

This workshop is a hands-on opportunity for attendees to become involved in re-enactments of multiple, fresh, bloody crime scenes without the worry of contaminating themselves or their clothing. Attendees will experience the “real thing,” be required to collect data, evaluate the scenes and statements of witnesses, determine the action resulting in the blood-letting events, determine potential classification of events, and conclude how these findings relate to subsequent criminal charges. This opportunity is closely followed by the bloodstain pattern analysis lecture, which, by its conclusion, will reveal the actions and events studied in the session workshop. The “scene” session is ideally limited to a maximum of 35 to 40 participants.

An opportunity to re-visit the scenes for re-evaluation purposes will occur at the close of the lecture session. The lecture session will be of immense value in the evaluation of the previously visited “scenes.” Such factors as position and movement of the victim, including removal and storage after the event, as well as bleeding prior to removal, can be determined. Estimation of volume loss and identification of tissue present can be utilized to determine the incident’s effect on the victim and, thus, his capacity for mobility under his own momentum. Actual analysis of blood is still a laboratory function; but the actions at the scene causing the blood loss are often of much greater value in reconstruction of the events of the incident that transpired than learning whose DNA is present. The physical relationship between individuals at the time of the incident can often be determined by bloodstain pattern analysis. Bloodstain patterns are classified by the type of action that produced them. Stain patterns reveal action that produced them and the amount of force required. Stain patterns reveal characteristics that can be utilized to determine directionality, number of impacts, and that actual position of the source at the time of the incident. Proper documentation of the scene, by either actual collection of items or by scene photography, allow the bloodstain pattern analysis to be completed at any distance and time. This analysis can be of value in determining events that have occurred and charges potentially brought against those individuals present during the event(s).

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#### **Hands-On, Bloodstain Patterns, Scene Significance**

### W19 Silent Disasters: Establishing and Operationalizing New Technologies for Missing Persons Programs

*Frederick R. Bieber, PhD\*, Harvard Medical School, Boston, MA; Charla Marshall, PhD\*, AFMES - AFDIL, Dover, DE; Chelsey Juarez, PhD\*, Fresno State University, Fresno, CA; Colleen Fitzpatrick, PhD\*, Identifinders International, Fountain Valley, CA; Bruce Budowle, PhD\*, University of Texas Health Science Center, Fort Worth, TX; Swathi A. Kumar, PhD\*, Verogen, San Diego, CA; Mayra Eduardoff, PhD\*, International Commission on Missing Persons, The Hague, Netherlands*

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**Learning Overview:** After attending this presentation, attendees will be more familiar with the established and emerging science, law, and ethics surrounding the use of emergent DNA technologies to bring justice and closure in the wake of genocide, armed conflict, and other humanitarian crises. This includes, but is not limited to, mitochondrial DNA, autosomal Single Nucleotide Polymorphisms (SNPs), and other non-traditional markers.

**Impact Statement:** This workshop will showcase innovative strategies and governance considerations for implementing missing persons programs and inform laboratories and organizations to scale these programs effectively.

Every year, 4,400 unidentified bodies are recovered in the United States, according to the National Missing and Unidentified Persons System (NamUs). National databases such as the Combined DNA Index System (CODIS) can provide a powerful framework for tracking repeat crime and confirming identity, yet they are utilized to identify approximately one percent of unidentified recovered remains. On an international level, statistics are lower, as most countries still do not have a national DNA index that can be leveraged to catalog and identify missing persons. The sheer number of remains that go unidentified from armed conflicts, acts of terrorism, and force majeure has collectively become a phenomenon known as the “silent mass disaster.”

This workshop will review how the community can use a multidisciplinary approach to successfully address this silent mass disaster. Experts from academia, policy specialists, forensic practitioners, social and forensic anthropologists, and genetic genealogy data analysts will review established and emerging methods to reconnect victims to families and to provide attendees with a blueprint for how they can operationalize an end-to-end small, large-scale or national program for the identification of unidentified human remains.

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#### Missing Persons, Kinship Studies, DNA Sequencing



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### W20 Novel Synthetic Opioids (NSO) in a Post-Fentanyl Analog Environment

*Donna Papsun, MS\*, NMS Labs, Horsham, PA; Reta Newman, MA\*, Pinellas County Forensic Lab, Largo, FL; Donna Iula, PhD\*, Cayman Chemical, Ann Arbor, MI; Michael Baumann, PhD\*, NIDA, Baltimore, MD; Robyn Parks, MD\*, County of Los Angeles Department of Medical Examiner-Coroner, Los Angeles, CA; Barry Logan, PhD\*, NMS Labs, Horsham, PA; Lynda Biedrzyski, MD\*, Waukesha County Medical Examiner Office, Waukesha, WI; Alex Krotulski, PhD\*, CFSRE, Willow Grove, PA; M.J. Menendez, JD\*, NMS Labs, Horsham, PA*

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**Learning Overview:** After attending this workshop, attendees will have a better understanding of how core-structure scheduling for fentanyl analogs and related drug policy actions have shifted the NSO landscape, what the new drugs on the markets are, and what challenges they pose to the scientific community.

**Impact Statement:** The presentations in this workshop will impact the forensic science community by providing an update covering the latest generation of NSO, providing comprehensive pharmacological and analytical characterization, drug chemistry and toxicology workflows, and case histories.

A new generation of NSO emerged after domestic class-wide scheduling of fentanyl-related substances in February of 2018 by the United States Drug Enforcement Administration followed by Chinese legislation that controlled two popular fentanyl precursor chemicals, NPP and 4-ANPP. These actions saw fentanyl analog positivity decreased for the most part; however, there was a subsequent shift to new varieties of opioid receptor agonists. These include 2-benzylbenzimidazoles (e.g., isotonitazene, metonitazene), benzimidazolones (e.g., bromphine), and cinnamoylpiperazines (e.g., 2-methyl AP-237, AP-238). The NPS monitoring community was able to use workflows honed over the past decade in response to these rapid market shifts to quickly identify these novel opioids, characterize them, and detect them in authentic toxicological specimens obtained during the course of medicolegal investigations, thus tying these new substances to impairment and harms.

This workshop will begin with a timeline of events, NSO trends, and an introduction of the tools and workflows that have proven effective in the quick identification of these new substances. Further, these novel opioids will be characterized, including discussions about development of standardized nomenclature and naming conventions, in vitro and in vivo studies, and postmortem cases that showcase the impacts posed by these substances. Later presentations will cover examples of how investigators and laboratories have mobilized to respond to the investigation of NPS toxicity outbreaks, and how the analytical data are used to support public health and safety measures. Two medical examiner perspectives from different parts of the country will be provided to share differing experiences and challenges with the novel synthetic opioids. The last part of the program will look at the impacts of open access information and timely dissemination. There will be discussion of strategies for early identification and monitoring proliferation of emerging substances and how this information lays the scientific support for scheduling actions. Finally, core-structure scheduling for fentanyl analogs will be reviewed from the perspective of how the NPS market responded, and if class-wide bans are considered a more effect drug policy strategy compared to specific drug scheduling actions.

Due to coordination, collaboration, and increased information sharing by the NPS monitoring community, the lifespans of newer novel synthetic opioids seem to be decreasing compared to previous substances, such as U-47700 and 2-furanylfentanyl. Although core-structure scheduling may have prompted new varieties of opioids, there appears to be decreased prevalence and a quicker response to those that ultimately emerge and proliferate. NSO will continue to impact the United States and its forensic science community. Collaborative efforts to combat these drugs are required and this workshop will be an example of that.

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#### Novel Synthetic Opioids, Novel Psychoactive Substances, Core-Structure Scheduling

### W21 The Forgotten Ones: Post-Traumatic Stress Disorder (PTSD) in First Responders

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**Learning Overview:** The goal of this presentation is to discuss various experiences of first responders from Emergency Medical Services (EMS), police, crime scene investigators, physicians, and others, in addition to different agency and regional responses to PTSD and processing of PTSD and grief. Symptoms experienced in these groups and how chronic exposure to trauma and vicarious trauma affect responders' mental health will be identified. This workshop will include small group discussions and larger collaborations of ways to prevent and recognize PTSD in one's home agency.

**Impact Statement:** This presentation will impact the forensic sciences community by addressing a serious aspect of mental health and responder well-being by discussing vicarious trauma, ways to recognize symptoms, and resources available for treatment.

This presentation will discuss how PTSD can affect first responders in federal, state, and local agencies such as local fire departments, law enforcement, emergency medical services, and public health officials. In this presentation, current research studies and data will be presented to help identify how PTSD is affecting first responders who are continuously exposed to traumatic and stressful incidents over the course of one's career.

PTSD and Acute Stress reactions are not reserved for crime victims and war veterans. The American Psychiatric Association defined PTSD during the 1980s in the Diagnostic and Statistical Manual of Mental Disorders, Third Edition.<sup>1</sup> Researchers have extensively researched the effects of PTSD on military veterans returning from conflict whereby mental health professionals were then able to use standard criteria to properly diagnose patients who have PTSD. Since the 1980s, researchers have been branching out from focusing on military veterans who have PTSD to researching the effects of PTSD on first responders.

The nation's first responders experience a wide variety of mental health symptoms that often go unrecognized. Recent research suggests that first responders such as firefighters, emergency medical technicians, paramedics, police officers, crime scene investigators, and allied health professionals are just as vulnerable to the effects PTSD. This presentation's focus will address the history of PTSD, recently published research, and new research areas to be studied in the future. For example, according to Stanley, the research data uncovered some shocking results of 1,027 retired and current firefighters, finding that 46.8% had suicidal ideations.<sup>2</sup> First responders have been overlooked over the years without the opportunity to ask for help. This can be attributed to the culture and stigmatism of being considered weak if one asked for mental health help to overcome the traumatic stress encountered on the job. Many first responders have suffered in silence, and the only way first responders will be able to get help is through further research into how PTSD affects first responders and public acknowledgment that PTSD is a real problem destroying first responder's lives.

This presentation is designed to help the forensic sciences community understand the serious implications of the current mental health crisis affecting first responders. Understanding how PTSD is affecting first responders may also help address the need to research the forensic science community's potential risk. The forensic science community is not immune to the effects of PTSD and should also investigate how PTSD could be affecting the members of the forensic science community.

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#### PTSD, Vicarious Trauma, PTSD-C

## A1 Pedagogical Access and Ethical Considerations in Forensic Anthropology and Bioarchaeology

*Micayla Spiros, MSc\*, Michigan State University, East Lansing, MI; Amber Piemons, MA, Michigan State University, East Lansing, MI; Jack Biggs, BA, Michigan State University, East Lansing, MI*

**Learning Overview:** After attending this presentation, attendees will have a pathway toward creating a digital pedagogy in forensic anthropology along with ethical considerations that must be made when developing online resources of skeletal materials. Attendees will also gain an understanding of the importance of equitable access to learning opportunities.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of developing a digital pedagogy to improve diversity and inclusion of our field through equitable resources.

Concepts such as evolution, environmental conditions, biological plasticity, and genetic theory are interdependent, deeply influenced by culture, and are the foundation of human variation. Human variation, being the pinnacle of osteological methodology, is critical for training osteologists. Thus, instruction must extend beyond “typical” models of human bones and generic osteological education. Using three online resources as case studies, this presentation will focus on the discussion of pedagogy, access, and ethics surrounding digital osteology. These three digital projects, 3D-MMS, MapMorph, and J-Skel, can be used to teach students topics ranging from human variation methods and theory to juvenile age estimation.

Digital pedagogy is the study of how to teach using digital resources. Technology can be used for in-classroom, hybrid, or fully online educational contexts. We can anticipate an increased expectation of digital technologies used for education and training following COVID-19. Traditional education in biological anthropology relies solely on hands-on and highly visual experiences. Therefore, forensic anthropologists are encouraged to collaborate in creating widespread digital pedagogy suitable for our discipline. This presentation discusses considerations and suggests pathways toward a forensic anthropology digital pedagogy, such as: accommodating for varying levels of digital fluency, equity in accessibility, ethical strategies, prioritization levels of content that need to be made publicly available, appropriate platforms and forms media for disseminating different types of content, and the necessity of multiple modalities.

While dialogue surrounding equity in higher education has gained momentum, discovering how to pursue awareness and then take action to rectify systemic issues of access still needs to be expanded.<sup>1</sup> Open-access to learning resources is one way to attract and retain students from a variety of backgrounds historically underrepresented in forensic anthropology. Skeletal and methodological casts are essential for learning but inaccessible in certain programs or remote learning. For research, skeletal collections have long been utilized to create, validate, and expand previous methods and protocols in higher education programs, yet research is dependent on the student’s location, opportunities for funding, and/or time constraints. Widespread academic access to donated skeletal materials also aims to eliminate the mistreatment and sharing of human remains that were unethically retained.

A clear avenue to accessibility is open-access paired with digital dissemination of pedagogical resources. Scholarship surrounding humans in any context should be equally tied to the education of ethical principles regarding the treatment of human remains. Legislation regarding how humans should be treated after death is slow moving and lacks the ability to stay current with constantly evolving pedagogical approaches; hence, the need for moral and ethical education. Key principles of ethics surrounding digital distribution of human remains include permission, respect, justification, and education.<sup>2</sup> When it comes to sharing human remains online, permission from the individual before death or from family members posthumously is the optimal standard. Likewise, the de-identification of donated remains and transparency from where and how the remains were obtained are essential. In bioarchaeological contexts, respect for the cultural beliefs and justification for education are necessary.

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2. D. Errickson; T.J.U Thompson. Sharing Is Not Always Caring: Social Media and the Dead. In: K. Squires, D. Errickson, N. Márquez-Grant (Eds.), *Ethical Approaches to Human Remains*, Springer, Cham, 2019, pp. 299-313.

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### Forensic Anthropology, Digital Pedagogy, Ethics

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## A2 A Virtual Anthropological Approach to Assessing the Similarity of Joint Surfaces With Applications for Sorting Commingled Human Remains

*Helen Brandt-Litavec, MS, Binghamton University, Binghamton, NY*

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**Learning Overview:** The learning objectives of this presentation are for attendees to develop a better understanding of the intra-/inter-individual variation at the sacroiliac joint and to recognize the utility in incorporating Virtual Anthropology (VA) methods when taking a quantitative approach to traditionally qualitative research.

**Impact Statement:** This presentation will impact the forensic community by providing an alternative method to sorting Commingled Human Remains (CHRs) using joint articulation analysis at the sacroiliac joint. This novel method will help improve current techniques for sorting CHRs.

CHRs complicate the identification process and can result in a significant loss of information regarding the individuals present. One common technique used to sort CHRs is joint articulation analysis. However, many current methods involve either physically fitting the bones together to determine goodness of fit or linear measurements. These techniques can be subjective and open to observer error or, in the case of linear measurements, limited in their ability to describe the entirety of the joint surfaces. In recent years, forensic researchers have demonstrated the ability to improve traditionally subjective techniques by incorporating VA methods. Therefore, this research aims to test the hypothesis that commingled individuals can be sorted through the comparison of their joint surfaces, specifically at the sacroiliac joint, by using VA methods.

Data were collected from individuals in the Binghamton University Skeletal Collection. The virtual models were created from 12 left os coxae and their articulating sacra using a NextEngine® 3D laser scanner. The shape of the auricular surfaces was analyzed using Landmark Editor and MorphoJ. Fifteen curves were placed around the exterior of the auricular surface and each curve was composed of three landmarks and eight semi-landmarks, resulting in a total of 135 landmarks.

Each individual's ilium and sacrum files were opened together in MorphoJ and a Procrustes analysis of fit analyzed the congruency of the two intra-individual auricular surfaces. The Procrustes sum of squares value produced by this test was recorded for each individual as a value of similarity. This value ranges from 0 to 1, with values closer to 0 indicating fewer differences in shape while values closer to 1 indicate extreme differences in shape. Subsequently, an artificially commingled sample of 104 of the possible combinations of commingled sacroiliac joints from the scanned virtual models was assembled to test the applicability of this method in separating CHRs. The same process was performed in MorphoJ, except with a random assortment of sacra and ilia files from separate individuals, and the Procrustes sum of squares values were also recorded for each commingled pair.

This work showed promising results. The Procrustes sum of squares values from sacroiliac joints of commingled individuals (mean=0.02108; range=0.00322 to 0.07038) are approximately twice as large as those of the non-commingled individuals (mean=0.01185; range=0.00198 to 0.02666). Additionally, the range and mean of the non-commingled group is closer to 0 than the artificially commingled group. Therefore, it appears that there is a noticeable difference between groups, in that the non-commingled individuals have more similarly shaped auricular surfaces than the commingled individuals. To understand if the perceived differences in the mean Procrustes sum of squares values between commingled and non-commingled groups are significantly different, an Independent Samples T-Test was conducted in IBM® SPSS Statistics 22. At the  $p < 0.05$  level, this test showed a significant difference between the commingled and non-commingled groups ( $p=0.026$ ).

In sum, these results illustrate the benefit of using VA methods to help sort CHRs at the sacroiliac joint. This novel and objective technique improves upon previously subjective strategies in sorting CHRs and in the future will be applied to additional joint surfaces.

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### Commingling, Virtual Anthropology, Sacroiliac Joint

## A3 The Segregation of Individuals From Large Commingled Assemblages Via Stable Isotope Ratio Analysis of Bone Collagen

Mary Megyesi, PhD\*, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI; Gregory Berg, PhD, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI; Lesley Chesson, MS, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI

**Learning Overview:** After attending this presentation, attendees will understand the application of intra-person isotopic variation limits for human bone collagen to segregate individuals from commingled assemblages.

**Impact Statement:** This presentation will impact the forensic science community by illustrating a process to resolve commingling using isotopic data that can be used when osteometric data are unavailable and DNA testing has limited success.

Stable isotope ratio analysis has been used by anthropologists since the 1970s to investigate diet. Human tissues, such as bone collagen, reflect the isotopic composition of food consumed. When diets vary due to food availability, distinct populations emerge isotopically; measuring the isotope “delta” values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in collagen from an unknown individual and comparing to previously characterized populations can thus be used to predict origin.<sup>1</sup>

Isotopic data could be useful for associating skeletal elements, as postulated by Berg et al. and McCormick et al.<sup>2,3</sup> Berg et al. published forensic interpretative values for: (1) determining if two bones “are probably” different (0.75‰ for  $\delta^{13}\text{C}$  values and 1.05‰ for  $\delta^{15}\text{N}$  values), and (2) determining if two bones “are” different (0.95‰ for  $\delta^{13}\text{C}$  values and 1.30‰ for  $\delta^{15}\text{N}$  values).<sup>2</sup> A model built by McCormick et al. with simulated isotopic and osteometric data confirmed the potential utility of stable isotope ratio analysis for resolving commingling.<sup>3</sup>

This study uses isotopic data gathered during casework at a skeletal identification laboratory to segregate individuals in commingled assemblages. Remains came from two Common Graves (CGs), exhumed from the Cabanatuan Prisoner of War Camp, Republic of the Philippines. A CG was used to bury all individuals who died within a 24-hour period. Historically, the CGs were associated with six (CG 1) and nine (CG 2) individuals. Remains were identified after World War II, leaving three unknowns from CG 1 and four unknowns from CG 2.

Collagen was extracted from 80 long bone samples and analyzed via elemental analyzer-isotope ratio mass spectrometry to measure  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Additional samples were collected for mitochondrial DNA (mtDNA) analysis. Chemical preservation hampered mtDNA results, with only 50% in CG 1 and 87% in CG 2 producing a sequence (typical=>95% success). Physical condition limited the use of osteometric methods, as most diaphyseal ends were eroded/missing.

Using the intra-person isotopic variation limits of Berg et al., four skeletal elements from CG 1 were segregated as originating from one individual.<sup>2</sup> All other samples overlapped in isotopic data distributions (i.e., formed one cluster). Between the remains of the three unknowns from CG 1, four mtDNA sequences were present; only one sequence was present in the elements segregated isotopically.

For CG 2, five isotopic data clusters were observed. Two isotopically distinct clusters represented single individuals, one consisting of 11 elements and the other of 3 elements. These isotopic segregations were supported by mtDNA data, as each contained a single sequence. Two additional clusters each contained a single mtDNA sequence but narrowly failed to meet the criteria for “are probably different.”<sup>2</sup> The largest isotopic data cluster ( $n=22$  elements) was associated with three mtDNA sequences. Overall, the data suggested the remains from CG 2 represented at least seven individuals, of which two could be segregated isotopically.

This study demonstrated that isotopic data can be used to segregate some individuals from commingled assemblages, even when population members were expected to have similar diets. Isotope testing alone is unlikely to resolve all commingling; however, once an assemblage is partially sorted, DNA analysis can be used strategically for deconvolution. Isotope testing is a useful tool in the forensic anthropologist’s toolbox that can be deployed in combination with other analytical techniques.

### Reference(s):

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### Stable Isotope Ratio Analysis, Bone Collagen, Commingling

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## A4 Forensic Anthropology Casework at the Cook County, Illinois, Medical Examiner's Office, 2012–2020

*Erin Waxenbaum, PhD\*, Northwestern University, Evanston, IL; Anne Grauer, PhD, Loyola University, Chicago, IL*

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for forensic anthropology consultations at the Cook County Illinois Medical Examiner's Office between 2012 and 2020.

**Impact Statement:** Attendees will appreciate forensic anthropology casework in the third- largest city in the country and be able to amass comparable data upon which decisions at the local, regional, and even national level can be informed.

As a relatively “young” area of forensic science, anthropology’s popularity in recent years has been fueled by media representations of the field, as well as greater numbers of medical examiners and coroner’s having first-hand interaction with forensic anthropology practitioners. However, few practicing forensic anthropologists publish on the composition of their casework, resulting in a minimal understanding by academics, forensic professionals, and lay individuals of the operationalization of the field. Many variables influence the number, type, and timeline of forensic anthropology casework in the United States, including local climate, jurisdiction caseload, and the familiarity of medical examiner/coroner’s offices with the field of forensic anthropology. Evaluation of the composition of anthropology casework from varied jurisdictions will provide essential data upon which planning, needs assessment, and resource determinations can be appraised. Continued discussion and publication of casework detail and composition not only serves future practitioners within anthropology but the broader audience of forensic scientists who engage with or request their expertise.

At the Cook County Illinois Medical Examiner’s Office, two anthropologists, working in concert, consult on a part time, case-by-case/as needed basis. The anthropologists are notified of a forensic pathologist’s consult after remains have been brought to the medical examiner’s office and, upon anthropologists’ requests, the remains are macerated, if necessary, by medical examiner’s staff.

With the objective of evaluating the composition of forensic anthropology casework at the Cook County Medical Examiner’s Office between 2012 and 2020, the following variables were recorded: month of discovery, decedent depositional environment, condition of remains upon initial discovery, type of anthropology consult requested, time from discovery to consultation request, time from consultation request to analysis, and time from analysis to report submission.

A total of 111 cases distributed over nine years produce an average of 12.33 cases per year, with the greatest number of cases occurring in 2015 ( $n=18$ ) and the fewest in 2020 ( $n=8$ ). A majority of cases were discovered in September ( $n=19$ ), with the fewest cases discovered in January ( $n=5$ ). Most cases were outdoor, surface recoveries ( $n=45$ ). Upon initial discovery, a majority of cases were fully skeletonized ( $n=48$ ), many were in a state of severe decomposition with partial skeletonization ( $n=30$ ), and few were fully fleshed ( $n=12$ ) or thermally altered ( $n=7$ ). While consult requests may include multiple components, 86 requests were for biological profile estimation, 51 requested trauma analysis, 15 requested human/non-human assessment, and 5 required the determination of the minimum number of individuals. On average, 90.7 days elapsed from discovery to anthropology consult, 51.0 days from consultation request to anthropology visit of the case, and 14.3 days from anthropology analysis in person to report submission.

Disclosure and discussion of the range of services, support, and expertise forensic anthropologists provide medical examiner and coroner’s offices broadens the understanding of the field, allows for the formulation of best practices, and serves as data upon which decisions regarding protocol, funding, resources, and need can be based. With additional practitioners collecting and sharing their data, a clearer window into the scope and utility of the field will be appreciated by both colleagues and the greater forensic scientific community.

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### Forensic Anthropology, Casework, Consultation

## A5 Myriad Maps and Applied Anthropology: Needs and Limitations

Molly Miranker, MA\*; Texas State University, San Marcos, TX

**Learning Overview:** The goal this presentation is to examine both the utility and limitations of different digital mapping techniques used in searches, rescues, recoveries, and tracking of missing person cases in a fatal migration context.

**Impact Statement:** The juxtaposition of different maps that depict searches for and the tracking of missing migrants specifically will impact the forensic science community by demonstrating how maps both facilitate inter-entity communication and can affect search or identification outcomes for human remains.

As the United States-Mexico border has become increasingly challenging to cross (ca. 1986), the number of migrant fatalities has proliferated in remote areas away from traditional ports of entry.<sup>1</sup> In the wake of these deaths, multiple federal, state, academic, and non-profit institutions, as well as affected families, have come together to search and rescue or recover and identify these decedents. Along with these efforts, maps and Global Positioning System (GPS) sharing have become common practice to launch search and recovery or maintain case tracking.<sup>2</sup> Currently, most maps that depict the numbers of deaths, dangers of terrain, or geospatial evidence for identification are based on Arizona data.<sup>1-3</sup> While the use and need for mapping is recognized in Texas, the data are, for now, insufficient for accomplishing similar spatial statistical analyses on a statewide scale.<sup>3,4</sup>

This contribution presents a contemporary sample of Google<sup>®</sup> Map imagery ( $n=2$ ), mapping apps traditionally used for hunting ( $n=2$ ), and Geographic Information Systems (GIS) -generated maps ( $n=2$ ) collected by this study over a field expedition during June–July 2021 in South Texas. This research was conducted under Institutional Review Board (IRB) approval and the maps were shared with interviewees' verbal consent. Specifically, the maps shared in this presentation were produced by law enforcement, firefighter/Emergency Medical Services (EMS), and non-profit organizations.

These exemplar maps reflect the ingenuity of those faced with a death crisis in Texas, as well as the barriers to maintaining sufficient information about missing migrant cases, specifically, inaccurate locational data and systemically decentralized forensically relevant information.<sup>5</sup> In combination, these long-standing obstacles prevent the establishment of an Open Geographic Information System (OGIS) in Texas that is similar to the Arizona OGIS. Without standardized data collection and maintenance in Texas, the risk of losing information and the inability to utilize spatial analyses for predictive capabilities and identification hypotheses will persist.<sup>4</sup>

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### Applied Anthropology, GIScience, Humanitarian GIS

## A6 Documenting Outdoor Simulated Scenes With Photogrammetry: Methods for Improving Dappled Lighting Conditions

*Caroline Jasiak, MA\*, University of Central Florida, Department of Anthropology, Orlando, FL; John Schultz, PhD, University of Central Florida, Department of Anthropology, Orlando, FL and the National Center for Forensic Science, University of Central Florida, Orlando, FL; Morgan Ferrell, MA, University of Central Florida, Department of Anthropology, Orlando, FL*

**Learning Overview:** After attending this presentation, attendees will understand how to combat adverse lighting conditions when employing Close-Range Photogrammetry (CRP) to record outdoor forensic scenes. In particular, this presentation will focus on using CRP methods to record two simulated scenarios representing variable outdoor lighting conditions.

**Impact Statement:** This presentation will advance forensic sciences by providing a preliminary investigation for mitigating adverse lighting conditions when recording scenes in outdoor environments. This research will improve previous photogrammetry research by exploring data collection techniques to improve imagery affected by adverse lighting conditions.

Although CRP is used to document archaeological sites and create 3D models, this method is not commonly employed to document forensic scenes and lacks a sufficient procedural basis to guide data recording when encountering problematic environmental conditions. The purpose of this study is to test how an overhead sheet and artificial lights can improve CRP imagery for scenes with adverse lighting conditions.

The scenes were located in the natural lands of the University of Central Florida and included mixed ground surfaces. Two scenes were created, one with trees partially blocking overhead sunlight and a second in an open location without tree cover. Scenes were constructed using a partial composite human teaching skeleton and clothing items to represent a slight scatter (approximately 4m x 3m). Calibrated photogrammetric scale bars from Cultural Heritage Imaging were placed around each scene, and they were photographed multiple times using various combinations of light correction tools: (1) no artificial lighting tools; (2) a white sheet over the scene; (3) artificial lights around the scene; and (4) a combination of lights and sheet. Approximately 240 photographs were taken for each model using a Nikon® D7200 camera, at five different view angles. Additional parallel overhead photographs and close-up photographs were also taken. The 3D models were processed multiple times using Agisoft® Metashape® Professional to improve the visual quality.

The 3D models of the two scenes were evaluated quantitatively and qualitatively. It is suggested that accurate models should have a Root Mean Square (RMS) reprojection error value of less than 0.3 pixels. Qualitative accuracy was assessed using orthomosaics and close-up model screengrabs of the cranium. The orthomosaic is an overhead view of the scene that is extracted from the 3D model. Assessing visual accuracy of the imagery involved subject-subject interface distortion, scale bar distortion, ground surface-subject distortion, and skeletal geometry distortion.

When comparing both scenes, the RMS reprojection error was highly accurate, as the error for all models was less than 0.3 pixels. While both scenes exhibited few qualitative distortions in the orthomosaics, the close-ups of the cranial screengrabs displayed increased skeletal geometry distortions. The dappled scene, without light correction tools, exhibited severe distortion due to bright sunlight patches. When the overhead sheet was utilized, these light patches were eliminated. However, scale bar distortion occurred where the overhead sheet provided minimal coverage. The open scene exhibited ground surface-subject interface distortion in all the orthomosaics. However, there was improved skeletal geometry accuracy of the close-up cranial screengrabs when the overhead sheet was applied. In both scenes, the use of the artificial lights did not appear to improve the quality of the 3D imagery.

It is recommended to use an overhead sheet, or light-colored tarp, when encountering dappled or overhead lighting issues when photographing forensic scenes for CRP applications. Proper cleaning of the scene prior to data collection is essential to improve model accuracy by removing all ground foliage around the skeletal elements and scale bars to reduce ground surface-subject matter distortions. Further research should involve larger scenes to develop protocols for implementing this recordation method.

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### Photogrammetry, Forensic Archeology, Scene Documentation



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## A7 Tracing Academic Relatedness Via The Forensic Anthropology Phylogeny (FAP)

*Katharine Kolpan, PhD, University of Idaho, Moscow, ID*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how the Forensic Anthropology Phylogeny (FAP) works and how it can provide information about academic relatedness across time and space.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how the FAP allows forensic anthropology practitioners to trace trends in academic relatedness over time and across institutions.

The purpose of this research is to introduce forensic practitioners to the FAP. The current iteration of the FAP was compiled via an ongoing volunteer survey. As such, it is meant to serve as a living document that is housed in cyberspace and periodically updated so that academic phylogenetic relationships can be tracked over time. The survey itself asks respondents to provide information concerning: their masters and doctoral institutions; their academic advisors; the years they matriculated and graduated; the type of degree earned (MA, MS, MSc, PhD); whether they specialized in any other subfield, such as bioarcheology, paleopathology, or molecular anthropology, while matriculating; any specific outside mentors who they felt aided their academic careers; their current employment; and any professional certifications they may hold. The survey also includes demographic information. The comprehensive nature of the survey questions is meant to provide information that can be reconstructed visually as a phylogenetic tree and to assess patterns of relationships between academic institutions via methods such as network analysis.

The FAP functions in a manner similar to that of the Academic Phylogeny of Biological Anthropology (APBA), but with a narrower focus and modifications meant to examine academic relationships that are specific to forensic anthropology. For example, the APBA phylogeny only allows for the participation of people who have completed their PhD. However, forensic anthropology has a significant applied component and attracts practitioners who make important contributions to the field without obtaining a doctorate. The FAP survey asks separate questions related to the completion of the master's degree and the PhD and maintains two phylogenetic trees, one for master's students and practitioners and one for PhDs. This creates a space in which to trace the academic trajectories of master's-level forensic anthropologists rather than erasing them. Moreover, as a highly specialized field with a limited number of doctoral institutions, many students of forensic anthropology who matriculate to the doctoral-level attend separate master's and PhD institutions. In this case, 52% of survey respondents attended disparate master's and PhD institutions, while 26% attended a combined MA/MS and PhD program at the same institution, and 22% attended a terminal master's program. Additionally, when students receive their master's and PhDs at different institutions, compiling data in two separate phylogenies allows for the analysis of relationships between master's and PhD institutions over time and space. For instance, of the seven respondents who attend the PhD program at Texas State, four completed their masters at New York University (NYU), while the other three respondents matriculated from different institutions.

Furthermore, because the phylogeny tracks a respondent's year of matriculation and graduation, the data capture trends related to which programs accept students from which institutions over time and permit researchers to examine patterns. The data from the phylogeny also capture the ebb and flow of programs, as well as when new programs begin to graduate students, where those students matriculate when and if they continue on with their education, and their current professional position. For instance, the four students attending Texas State with master's degrees from NYU all matriculated after 2008, which makes sense since NYU did not institute its Master of Science in Human Skeletal Biology until the mid-2000s and Texas State's PhD in Applied Anthropology program is quite new.

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### Phylogenetics, Academia, Network Analysis

## A8 Exploring Forensic Research Interests of a United States-Based Anthropology Faculty

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how forensic anthropological research interests are presented by forensic and biological anthropologists.

**Impact Statement:** This presentation will impact the forensic science community by examining the inclusion of forensic anthropology as a research interest by individuals in the broader field of biological anthropology.

Forensic anthropology is commonly considered a subdiscipline of biological anthropology. While the discipline of forensic anthropology currently requires specific training and certification, many faculty members in the broader discipline of biological anthropology continue to list forensic anthropology, or “forensics,” as a research interest, specialty, or focus (herein referred to as research interest) in addition to their primary research foci. This project aims to investigate whether those who list forensic anthropology as a research interest are invested in the discipline of forensic anthropology.

As of 2021, the Integrated Postsecondary Education Data System lists 459 schools that offer undergraduate anthropology degrees in the United States. Of these, 215 schools were found to have public departmental websites where faculty reported research interests. Pertinent data (e.g., research interests) were webscraped, culminating in 2,831 total faculty profiles. Of these, 78 individuals (2.8% of all faculty) listed some variation of “forensic” as a research interest. For the purposes of this study, individuals were categorized into forensic and non-forensic groups. Individuals were placed into the forensic group if one of two criteria were met: (1) certification through the American Board of Forensic Anthropology; and/or (2) active membership in the American Academy of Forensic Sciences (AAFS) conference.

Of the 78 individuals, 42 (53.8%) were designated to the forensic group and 36 (46.2%) were designated to the non-forensic group. In regard to active research in the discipline, 88.1% of the forensic faculty, have presented at least once at an annual meeting of the AAFS and 73.8% of forensic faculty have published at least one paper in the *Journal of Forensic Sciences (JFS)*. On the other hand, only 2.8% of the non-forensic faculty have presented at the AAFS, and only 16.7% of the non-forensic faculty have published in the *JFS*.

Research interests were also examined to reveal whether forensic faculty were more likely to list specialties within forensic anthropology (e.g., age estimation, trauma); 52.4% of forensic faculty and 13.9% of non-forensic faculty listed specific forensic anthropological specialties. This was further explored via the use of word frequencies and predictors (via term frequency-inverse document frequency [tf-idf] scores) in R using the “tidytext” package. Words more predictive of forensic faculty include “bone,” “methods,” “death,” “growth,” and “quantitative,” while words more predictive of non-forensic faculty include “studies,” “southwest,” “southeast,” “paleoanthropology,” and “identity.” These word predictors indicate a methodological focus among the forensic faculty and more regional and/or theoretical foci among the non-forensic faculty. However, when clustered by natural topics in R using the “topicmodels” package, individuals could not be reliably classified into the forensic and non-forensic groups.

Finally, Network Analysis procedures in R using the “textnet” and “igraph” packages assessed latent social structures among and between the two groups. These analyses highlight trends seen in the word frequencies and predictors, but better showcase the relationship between research word and forensic status, as well as latent relationships among all faculty. While several words are common in both groups (e.g., anthropology, forensic, bioarcheology), words such as “legal,” “identification,” “histology,” and “biomechanics” are more likely to be used by forensic faculty and words such as “cultural,” “theory,” “mortuary,” and “paleoanthropology” are more likely to be used by non-forensic faculty. This network highlights areas of overlap and divergence in research between forensic anthropology and the greater biological anthropology.

### Forensic Anthropology, Text Analysis, Network Analysis

## A9 Advances in Sorting Commingled Human Remains: A Preliminary Study Using the Subtalar Joint

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the issue of commingled human remains and how geometric morphometrics target to solve it.

**Impact Statement:** This presentation will impact the forensic science community by presenting a new semi-automated approach in re-associating commingled human remains.

The term commingling is used for characterizing the osteological contexts in which skeletal remains of different origins are intermixed.<sup>1</sup> Its presence is common in both forensic and bioarcheological contexts and, until recently, the sorting methods were mainly based on optical similarities or metric re-association.<sup>2</sup> Given that multiple skeletal elements may present optical similarities due to the impact of the same taphonomic factors, as well as multiple skeletal elements may be of similar size, the evaluation of shape may be more individualized.

For the evaluation of shape, the tali and calcanei of 25 individuals were scanned with a structured light 3D scanner. The individuals originate from a skeletal collection that is housed at the Forensic Anthropology Unit, Department of Forensic Medicine and Toxicology, School of Medicine, National and Kapodistrian University of Athens. A number of six landmarks were placed on the rim of the talus' posterior articular surface for the calcaneus and the adjoining calcaneus' posterior articular surface for the talus. The landmarks' raw coordinates were transformed into Procrustes coordinates for removing the factor of size. The two skeletal elements that presented the smallest Procrustes distance were the ones that mostly resembled in overall shape and it was recorded whether they actually belonged to the same individual.

The results showed that in 22/25 (88.00%) of the cases, the correct talus-calcaneus match was found between the skeletal elements that presented the two smallest Procrustes distances compared with all the other elements. It is advised that this method should be utilized in rejecting an approximate 90% of the possible matches based on shape differences.

Future research could further evaluate the applicability of landmark and/or semi-landmark-based methods in re-associating two bones with adjoining articular surfaces.<sup>3</sup> The aforementioned approach could retrieve shape information for each skeletal element of the articulation and become a valuable addition to the existing sorting methods.

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### Forensic Anthropology, Commingling, Sorting

## A10 Calculating the Minimum Number of Individuals (MNI) and Most Likely Number of Individuals (MLNI) for a Commingled Fetal and Infant Remains Sample

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**Learning Overview:** After attending this presentation, attendees will be informed about how to calculate both the MNI and the MLNI of commingled fetal and infant remains.

**Impact Statement:** This presentation will provide information about how to investigate commingling in fetal and infant remains, as there is scant literature that discusses resolution for this group of individuals.

Various taphonomic forces can cause disarticulation of human remains; as a result, forensic anthropologists are often tasked with separating remains for individualization purposes. Literature on the commingling of infant bones has not been as developed when compared to that regarding the commingling of adult bones. Therefore, this research project focuses on estimating MNI and MLNI in infant and fetal remains.

Fetal and infant remains comprising part of the Antioquia Modern Skeletal Reference Collection were examined. This assemblage is a commingled one from a modern ossuary and therefore represents a real-world case study of commingling in fetal and infant remains. Every region of the body is represented with an absolute number of 271 bones present in the assemblage. The side, estimated age based on development and metrics, and other notable characteristics, such as taphonomic alterations, were recorded for each bone. Schaefer et al. was used for measurement definitions as well as to estimate age.<sup>1</sup> Linear measurements were taken in millimeters (mm) using a digital sliding caliper. Age was estimated when possible for each element based on the metrics, and this study used this age division when calculating MNI and MLNI. The MNI was calculated based on the formula:  $N = \text{Max}(L, R)$ .<sup>2</sup> This is done by sorting the assemblage into bone type, then left and right sides; the bone and side with the most elements are recorded as the MNI. The MLNI was calculated to ensure that the number of individuals were accurately estimated. The literature suggests this metric is a better estimate of the number of individuals because pair matching is taken into account, especially for well-preserved samples.<sup>3</sup> Here, the long bones (humeri, radii, ulnae, femora, tibiae, and fibulae) were used to estimate MLNI because they have a stronger pair-wise association than other bones.<sup>3</sup> The Chapman formula,  $N = ((R+1)(L+1) / (P+1)) - 1$ , was used to calculate MLNI.<sup>4</sup> Visual pair matching (color, staining, morphology, etc.) coupled with age estimation from metrics was used to match antimeres of long bones.

The estimated ages fell into nine separate cohorts from 22 prenatal weeks to 40+ weeks. Taking these ages into account, the calculated MLNI of the long bones ranged from 2–20, while the MNI of the same bones ranged from 2–7. The range for MLNI is greater than that for MNI; this may be due to the relatively small number of pairs (16) of long bones found in the assemblage. Bones falling into the 40 prenatal weeks and 40+ weeks age cohorts have the largest MNI and are the most represented in the sample.

Resolving commingling in fetal and infant remains can be more complex than for adult remains due to the strong effect that small differences in age (i.e., on the order of single months) will have on size. Their fragility as compared to adult remains can also hamper recovery and measurement efforts. As a result, this research aims to provide practical procedures involving the commingling of infant bones and reporting MNI vs. MLNI. In the case of infant and fetal remains, this study recommends the use of MLNI over MNI as a stronger metric for resolving commingling.

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### Infant Remains, Commingling, MNI

## A11 We Take Care of Our Own: Utilizing the LGBT+ Accountability for Missing and Murdered Persons (LAMMP) Database

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**Learning Overview:** Attendees will understand the need for a queer-informed database for missing and murdered LGBTQ2S+ (Lesbian, Gay, Bisexual, Transgender, Queer, Two Spirit) community members and will learn how to use and contribute to this database. Attendees will also understand the limitations of traditional databases in the context of increasing fatal violence directed at LGBTQ2S+ people.

**Impact Statement:** A variety of resources are needed to document LGBTQ2S+ decedents who may not be accurately reported in traditional databases. This presentation offers suggestions for the use of a new database to expediently track cases, identify decedent patterns, and share essential information leading to identification.

Rising fatal violence and hate crimes against LGBTQ2S+ individuals necessitates a more strategic response outside of existing databases. The most recent Federal Bureau of Investigation (FBI) data on hate crimes (2019) indicated that 20.3% of incidents were committed due to sexual orientation or gender identity.<sup>1</sup> Government databases and clearinghouses such as the National Missing and Unidentified Persons System (NamUs) and National Center for Missing and Exploited Children (NCMEC), along with public entities like Charley Project (CP) and Doe Network (DN), are powerful tools for use in information sharing about missing and murdered individuals. Yet, these and other databases and state clearinghouses present challenges when researching or entering cases of LGBTQ2S+ individuals.

For example, missing and unidentified persons databases lack clear guidelines for entry of inconclusive or uncertain sex, exclude selections outside binary sex, and lack input options for evidence of gender identity. This can both lead to biases and gaps in functionality within databases, thus making it more difficult to recognize such biases. Further, deadnames (the individual's name given at birth that is no longer used) are often entered and pre-transition photos are often used in media and databases, while their current names and photos may be excluded. Misgendering, deadnaming, and focusing on the individual's assigned sex potentially exposes the individual to further harm and hinders case resolution.<sup>2</sup>

In response to inadequate databases, the LGBT+ Accountability for Missing and Murdered Persons (LAMMP) database was created to gather data with queer-informed and harm-reduction perspectives.<sup>3</sup> LAMMP acts as a citizen-powered resource to serve the LGBTQ2S+ community by: (1) matching unidentified, possibly LGBTQ2S+ Does with missing persons reports; (2) identifying cases of possibly LGBTQ2S+ individuals based on available information; and (3) providing a comprehensive, searchable database for family members (chosen family and/or family of origin), law enforcement, and others to provide essential information leading toward identification.

LAMMP offers several features to ensure the privacy and safety of the missing and those reporting them. Case reports and information can be submitted to administrators anonymously and without creating an account. The missing persons component of LAMMP is private and searchable only to those with approved credentials. The database is intended for use by professional investigators and researchers (e.g., anthropologists, medical examiners, law enforcement), as well as forensic genetic genealogists, and includes fields for GEDMatch numbers of family members and related family trees.

This presentation will present LAMMP case examples to demonstrate how the database differs from NamUs, NCMEC, DN, CP, and other clearinghouses in the following ways: (1) incorporation of data from non-traditional sources (e.g., Facebook® posts, Reddit® threads, activist organizing); (2) removal of the gatekeeping aspect of governmental databases as information does not have to be submitted by law enforcement, forensic professionals, or reported by family of origin; and (3) recognition of, and response to, the problems caused by binary sorting of individuals in databases, allowing for comparison across sex and gender categories.

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- <sup>3</sup> Trans Doe Task Force. *LGBT+ Accountability for Missing and Murdered Persons (LAMMP) Database*. Retrieved 8/19/21, <http://lammp.transdoetaskforce.org/>.

### Identification, LGBTQ+, Database

## A12 Applying a Biocultural Signature of Embodied Stress to Identify Latin American Migrants from a Cold Case Sample in South Florida

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**Learning Overview:** After attending this presentation, attendees will understand how skeletal and dental indicators of stress may be used to recognize the skeletal remains of Latin American migrants in the South Florida region.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence supporting the inclusion of skeletal and dental indicators of non-specific stress in the biocultural profile of Latin American migrants in South Florida.

Immigration policy in the United States remains a polarizing topic of discussion throughout the political spectrum. Current policy amendments making immigration to the United States illegal or more difficult contribute to the ever-rising number of undocumented migrants who have died while attempting to enter the United States through rural and dangerous regions—such as southern Arizona’s remote and inhospitable Sonoran Desert and mountains.

Compared with migrants crossing the United States-Mexico border, less forensic anthropological attention has been paid to migrants entering South Florida.<sup>1</sup> The difficulties associated with aquatic deposition of remains make the recovery of migrants’ remains from the South Florida coastline challenging. The diverse cultural and geographic origins and multifaceted biological histories of Latin American populations in the South Florida region—especially Cuban Americans—also make discriminant function analyses based on traditional craniometrics inadequate for estimation of population affinity or region of origin.<sup>2-4</sup>

A forensic anthropologist’s ability to differentiate between the remains of Latin American migrants and those of United States-born citizens is essential to forensic investigations. This study expands on previous research conducted by forensic anthropologists on the southwestern United States border and tests its relevance to the unique context of the South Florida “border.”<sup>4</sup> A skeletal sample of 102 cold-case decedents in the Miami-Dade County Medical Examiner Department was analyzed for the presence of skeletal and dental indicators of non-specific stress, particularly porotic cranial lesions and enamel hypoplasias, in an attempt to flag decedents as possible migrants and potentially guide the investigation of their cases.

Of the 102 decedents, 28 were identified or had believed-to-be associations; these 28 served as the control group of known origin (i.e., United States- vs. Latin American-born.). Of the control sample, 46% ( $n=13$ ) of decedents had enamel hypoplasias, which were over three times more likely in Latin American migrants ( $n=10$ ) than United States-born citizens ( $n=3$ ). Of the control sample, 18% ( $n=5$ ) had porotic cranial lesions, which were four times more likely in Latin American migrants ( $n=4$ ) than in United States-born citizens ( $n=1$ ).

These data suggest that the stress indicators that have proved effective in differentiating United States from non-United States decedents in the southwest may also be applicable to the context of South Florida casework. Thus, the indicators of stress were examined in the sample’s 74 unidentified decedents. Of these, 24% ( $n=18$ ) had enamel hypoplasias, and 23% ( $n=17$ ) had porotic cranial lesions. Based on the presence of these stress indicators, 28 sets of unidentified skeletal remains were flagged as possible migrants. It is hoped that these tentative associations inform future case investigation and expedite identifications for these individuals.

The above findings are consistent with documented inequities between many Latin American countries and the United States in access to health care and a nutritive diet at the time of growth and development. The presence of non-specific indicators of stress, in combination with context and other aspects of the biocultural profile, should indicate to forensic anthropologists and other medicolegal personnel that the remains in question may belong to Latin American migrants—not only in the southwest, but also in the region of South Florida.<sup>5</sup>

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### Forensic Anthropology, Unidentified Migrants, Non-Specific Stress Indicators

## A13 OsteoID: A Freely Available Tool for Skeletal Species Identification

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**Learning Overview:** After attending this presentation, attendees will be familiar with a new online resource that facilitates species identification from skeletal remains.

**Impact Statement:** This presentation will impact the forensic science community by introducing a freely available online tool that can be utilized by medicolegal agencies to evaluate forensic significance of skeletal remains.

Without specific training in comparative osteology, determining whether an isolated skeletal element is human or faunal in origin can be challenging. Many medicolegal agencies will rely on forensic anthropologists to make such assessments, and although forensic anthropologists are experts on the human skeleton, they may rely on other resources to determine the specific species of isolated faunal elements. Not all forensic anthropologists or medicolegal agencies have access to large comparative skeletal collections and may rely on textbooks or broad internet searches. The goal of this project was to create a freely available online tool to facilitate species identification from skeletal remains.

Photographs and metric data were collected from the long bones, scapulae, sacra, and ossa coxae of 28 common North American species, including humans, 20 additional mammals, three birds, and two turtles. In total, more than 16,000 measurements were collected from 436 human and 984 non-human specimens. The photographs were edited to create a single figure with multiple views for each element (six views for all long bones), along with a metric scale, penny for scale, and documented range of maximum bone length. At times, unique identifying features are also indicated on the figures. Descriptive statistics were run on the metric data, which consisted of maximum bone lengths, midshaft diameters, and proximal and distal breadths and depths. These resources were then incorporated into an online tool: OsteoID.<sup>1</sup>

OsteoID asks users to first identify what type of element (i.e., bone) they have, providing exemplar images of the various forms of each bone; if unknown, users can search all elements. Users can then input the maximum length, proximal width, or distal width of the bone (one at a time in a nested format), which will return images for only those species within that size range. The search tool was limited to these three measurements because they could be collected from almost all element types and preliminary research indicated they were the easiest for less-experienced users to collect. Size ranges input into the tool were based either on the full measurement range from the data or two-standard deviations from the mean, whichever was broader to better encompass size variations. Clicking on the figures enlarges them for visual comparison and species identification. Additional resources are also provided at the website, including a link to a Morphosource project page where 3D scans of many of the elements have been uploaded as part of this study, a link to access images of other non-measured elements (e.g., crania, carpals and tarsals), and a list of other texts or webpages that may be of use to practitioners.<sup>2</sup> OsteoID is now publicly available, providing an additional and free resource to forensic anthropologists, medicolegal agencies, and archaeologists, among others. Research is ongoing to assess the possibility of including a more robust statistical framework for species identification, although all final identifications should be confirmed via visual assessments.

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2. Morphosource. [www.morphosource.org](http://www.morphosource.org) (accessed August 27, 2021).

### Forensic Anthropology, Forensic Significance, Non-Human

## A14 Assessing Opioid Exposure and Opioid Overdose Deaths in Skeletal Casework Through Collaboration With Organ Procurement Organizations

Janna Andronowski, PhD, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; Randi Depp, MS\*, The University of Akron, Akron, OH

**Learning Overview:** Attendees of this presentation will be introduced to critical information relating to the effects of prolonged opioid use on skeletal biology through modern decedent data collected in collaboration with an American Organ Procurement Organization (OPO). Specific goals include: (1) outlining the lack of detailed decedent medical and social histories in existing skeletal collections commonly used in forensic anthropology, and (2) demonstrating the effectiveness of targeted skeletal specimen procurement through medical-based organizations.

**Impact Statement:** This presentation will impact the forensic science community by providing new insights and potential avenues for modern skeletal sample procurement for forensic anthropological research. Accessing well-documented modern specimen collections will allow us to adapt morphological and histologic methods within the field of forensic anthropology for application to chronic opioid users.

The opioid epidemic is a public health crisis that resulted in almost 50,000 Opioid Overdose Deaths (OODs) in 2019 alone.<sup>1</sup> Exacerbated by the COVID-19 pandemic, OODs jumped to more than 69,000 in 2020.<sup>2</sup> Commonly encountered in forensic casework, chronic opioid use and OODs pose a serious problem for forensic anthropological analyses due to induced osteoporotic effects demonstrated in clinical research.<sup>3</sup> As such, it is vital that future research seeks to understand the impact of substance abuse on forensic anthropological methodologies. Existing human skeletal collections, however, are not wholly representative of modern populations and drug users, and detailed drug use histories are especially lacking.

To fill this gap, it is proposed that researchers utilize OPOs to target modern populations, especially marginalized and underrepresented groups, including OODs. Demographics of 100 recent decedents from Northeast Ohio were obtained through an American OPO and compared to the demographics of existing skeletal collections (William M. Bass Donated Skeletal Collection, Hamann-Todd Human Osteological Collection, and Robert J. Terry Anatomical Collection). The Bass and Terry collections have similar sex distributions as the Northeast Ohio samples with approximately 60% males and 40% females. The Hamann-Todd collection contains predominantly males (83%). The Northeast Ohio samples were more balanced for age distribution, with nearly equal distribution for age cohorts 20-29 (22%), 30-39 (23%), 40-49 (27%), and 50-59 (25%). All three skeletal collections had left-skewed age distributions with middle-aged and older cohorts represented more frequently. Ancestral affinity was not representative of larger populations for either Northeast Ohio samples or skeletal collections, but this may be more reflective of historical mistrust in the medical community by minority groups.<sup>4</sup> Still, decedents from Northeast Ohio included 80% European ancestry and 10% each of African American and Latinx ancestries.

The detailed medical and social histories of our Northeast Ohio decedents illustrated that demographic risk factors often interacted (e.g., prior incarceration, chronic health conditions, mental health disorders) and many OODs have multiple marginalized identities and/or contributing health cofactors. Of the 100 decedents, 17 were OODs and accounted for 41% of those previously incarcerated and all LGBTQIA+ individuals. Of the OODs, 53% involved polysubstance use, 41% had chronic health conditions (e.g., high blood pressure, diabetes, chronic pain, HCV, HBV, HIV), and 47% had a known mental health disorder(s).

Medically based sources (e.g., OPOs) are ideal for skeletal research since their extensive medical histories reveal many comorbidities and lifestyle factors that are typically unknown when studying traditional skeletal collections and human remains curated via body donation programs. Overall, the Northeast Ohio data demonstrate the effectiveness of OPOs to provide skeletal samples that are more representative of targeted modern groups under study, particularly when a balanced age distribution is desired.

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2. Ahmad, F.; Rossen, L.; Sutton, P. *Provisional Drug Overdose Death Counts*. National Center for Health Statistics, 2021.
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### Opioids, Forensic Anthropology, Skeletal Collections



## A15 Evaluating the Effect of Cocaine Use on Dorsum Sellae (DS) Demineralization

Tanin Zadeh, MS\*, Western University of Health Sciences, Pomona, CA; Ellen Fricano, PhD, Western University of Health Sciences, Pomona, CA; Vicki Wedel, PhD, Western University of Health Sciences, Pomona, CA

**Learning Overview:** After attending this presentation, attendees will have developed a better understanding of the relationship between cocaine use and dorsum sellae demineralization.

**Impact Statement:** This presentation will impact the forensic community by demonstrating the value of identifying the correlates of cranial bone resorption amongst multiple variables.

**Introduction:** Bone metabolism is normally under control due to a balance between osteoblastic and osteoclastic activity, but when one process exceeds the other, bone growth or destruction result. The Sella Turcica (ST) is a depression in the sphenoid in the base of the skull that borders many important anatomical structures including the pituitary gland in the hypophyseal fossa, internal carotid and anterior cerebral arteries, cavernous sinus, and cranial nerves. The Dorsum Sella (DS) makes up the posterior wall of the ST and is bounded by the posterior clinoid processes laterally. Of the cranial bones frequently eroded, the DS is at great risk due to its thin nature, with an average thickness of 2-7mm. This study aimed to explore whether a correlational relationship exists between cocaine use and DS Demineralization (DSD), with other potential correlates, such as age, alcohol use, hypertension, and diabetes, being tested as well.

**Hypotheses:** H<sub>1</sub>: There is significantly more DSD in cocaine users than in non-users. H<sub>2</sub>: Additional variables that could result in DSD are age, sex, hypertension, diabetes, and alcohol use.

**Materials and Methods:** Computed Tomography (CT) scans of 201 individuals ( $n=109$  females,  $n=92$  males) were compiled from the New Mexico Decedent Image Database (NMDID). Medical records indicate 108 had history of cocaine abuse and 93 did not. Scans were analyzed using Dragonfly in which a contour mesh of bone was created to visualize a 3D model (slice thickness=1mm with 0.5mm overlap). The DS was assessed to locate any perforations indicating DSD. Statistical analysis was performed using chi squared analysis and binomial logistic regression.

**Results:** Of those who reportedly used cocaine, 20 had perforations and 87 did not. Out of the non-users, 30 had perforations while 61 did not. It was found that a reported history of cocaine use was not correlated with DSD in this sample and the frequency of DSD was higher in the non-user group. Using a multivariate binomial logistic regression, it was found that the relationship between DSD and age, sex, hypertension, diabetes, and alcohol use was found to be significant ( $p<0.001$ ). This model explains 13% of the variation in DSD found. However, only age ( $p<0.001$ ) and sex ( $p=0.05$ ) were found to be driving this significant relationship with DSD. DSD is more frequently present in older and female individuals.

**Discussion:** Per research, this study was the first to utilize CT imaging to assess the frequency of DSD while accounting for environmental and health factors. It was demonstrated that DSD may be more common in the broader population than previously thought. The hypothesis that suggested a correlation between DSD and cocaine use was not supported; however, there are a number of potentially confounding factors that may be obscuring the relationship. Most importantly, there were no records of duration or volume of cocaine use, method of ingestion, or purity of the cocaine.

The only risk factors for DSD that were significantly correlated were both natural, physiologic processes. On average, the non-user group included older individuals, which may be a critical factor considering the effect of natural bone loss with age. In addition, females have overall thinner bone structure than males and lose bone density with age and menopause as well.

**Significance:** Assessing the frequency of DSD in cocaine users and the general population is valuable to the forensic community due to the wide range of physiologic and pathologic processes that result in bone demineralization. This study can be used as a foundation for utilizing radiography to ID unidentified remains by matching physical findings to changes expected from medical and social histories.

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### Sella Turcica, Bone Resorption, Computed Tomography (CT)

**A16 The Effects of Prolonged Opioid Exposure on Bone Fracture Strength of Rabbit Middle Ribs**

Reed Davis, MSc\*, The University of Akron, Akron, OH; Mary Cole, PhD, The Ohio State University, Columbus, OH; Henry Astley, PhD, The University of Akron, Akron, OH; Janna Andronowski PhD, Memorial University of Newfoundland, St. John's, NL, Canada

**Learning Overview:** After attending this presentation, attendees will better understand the effects of prolonged opioid use on bone mechanical qualities.

**Impact Statement:** This presentation impacts the forensic science community by demonstrating the differences in force required to fracture the middle ribs of a model organism. These data may serve as a foundation for examining the effects of opioids on human bone fracture in a forensic context.

Previous work on the effects of opioids on bone maintenance and healing have focused on weight-bearing bones, such as the spine, pelvis, and femur.<sup>1,2</sup> Studies examining non-weight-bearing bones have focused on medical record analyses to predict future fracture risk, but none explicitly examine the effects of opioid use on bone mechanical properties and fracture risk.<sup>3</sup> Examining changes in yield strength of rib segments collected from rabbits exposed to opioid analgesics demonstrates how prolonged opioid use changes bone mechanical properties. Ribs were selected as they are under relatively constant stress from respiration and do not bear body weight, thus limiting external effects from lifestyle. The middle ribs are a common site used for histologic aging in forensic anthropology.<sup>4,5</sup> Murine models (mice and rats) require cortical bone remodeling to be induced. As such, rabbits were chosen as a model organism since they are the smallest, commonly used laboratory animal with spontaneous cortical bone remodeling similar to humans. This experiment sought to determine if: (1) opioid use causes changes in bone geometry of non-weight-bearing bones; and (2) biomechanical properties (e.g., peak stress, elastic modulus) of opioid groups are lower compared to controls examined via compression testing.

Rabbits were divided into three groups (fentanyl, morphine, and control). Differences in drug administration required the controls to be split into two groups. Following eight weeks of morphine/saline injections or transdermal delivery of fentanyl/sham patch, animals were euthanized. Right fifth ribs were excised from each animal and cleaned of soft tissue and the ventral portions were sectioned into thirds for testing. Each sample was tested until failure using an Instron® 5567 universal test frame housed at Akron Polymer Training Services using a 1kN load cell and a compression rate of 0.05mm/min. Data were normalized by cortical area and sample length of the rib section, respectively, to produce stress-strain curves and analyzed using MATLAB® (R2021a, Mathworks). Gross rib measurements were collected before sectioning and compared using the BoneJ plugin in Fiji.<sup>6,7</sup> Statistical analyses were completed using a one-way Analysis of Variance (ANOVA) using drug treatment as a fixed factor in SPSS (v.26, IBM®).

Preliminary results indicated that drug group animals were not significantly different from controls in either the gross geometric or biomechanical properties tested. Due to differing drug administration routes and splitting of the control group to accommodate this, the sample size was uneven, which may have contributed to a lack of statistical power. Additional statistical analyses are planned to account for individual differences between animals. Certain animals in this study further adapted their behavior to compensate for physiological changes caused by opioid administration (e.g., lethargy), which may have limited observed differences between groups.

Per research, this work demonstrates the first experimental method of assessing fracture risk from opioid use in non-weight-bearing bones. Future work may apply this method to forensic samples to determine what role, if any, opioids played in fracture risk.

*Certain work included here is supported by the National Institute of Justice (Award 2018-DU-BX-0188). The opinions, findings, and conclusions expressed are those of the authors and do not necessarily reflect those of the Department of Justice.*

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**Skeletal Biology, Biomechanics, Opioid Use**

## A17 Understanding Skeletal and Body Donation in Forensic Anthropology: Developments and Procedures

Kerianne Armelli, MS, Kent State University, Kent, OH; Rhian Dunn, MS, Michigan State University, East Lansing, MI; Carolyn Isaac, PhD, Michigan State University, East Lansing, MI

**Learning Overview:** The results of this presentation will reflect and compare the current practices of skeletal and body donation within forensic anthropology for attendees, both in the United States and worldwide.

**Impact Statement:** This presentation will impact the forensic science community by initiating discussions about the practice of body donation in forensic anthropology and guidelines the discipline wants to establish for developing and maintaining skeletal collections for teaching and research.

Donation programs have been an indispensable component of medical education, where human anatomy has benefited from dissection and laboratory coursework.<sup>1</sup> In recent years, donation programs have also become an integral component within anthropology programs, where skeletal donations are an important tool for study and research.<sup>2,3</sup> Yet the manner in which skeletal materials are obtained and maintained varies between programs. Currently no protocols have been established regarding the management and curation of a donor skeletal collection within forensic anthropology.

The purpose of this research is to document and compare current practices and opinions regarding the development and maintenance of body donation programs and skeletal collections in forensic anthropology. Two anonymous surveys were electronically distributed to the forensic anthropology community. The first survey focused on practitioner's opinions of body donation within forensic anthropology and the second survey explored current practices in body donation.

There were 111 participants, including individuals from Asia (1.2%), Europe (4.9%), and Africa (1.2%), although the majority were from North America (92.7%) with most employed within a university setting (79.3%). It was found that skeletal collections are most commonly used to teach undergraduates (59.3%), followed by masters and doctoral students (48.1% and 43.2%, respectively). Respondents indicated that skeletal casts (63%) and purchased human skeletal materials (50.6%) were most commonly used.

Almost all of the survey respondents (95.1%) agreed with the use of donated materials for teaching and research, but agreement was less for the use of historic (48.8%), unclaimed (38.3%), or unidentified (32.9%) individuals, and only 26.8% thought that unprovenanced archaeological remains should be used. The majority of respondents (89%) were also in favor of accepting donations by next-of-kin, but fewer respondents agreed with the acceptance of donations from private citizens (59.8%), medical examiners/coroners (46.9%), or skeletal material found on private property (24.7%). Utilizing donors in trauma or taphonomic research was acceptable to 70.4% and 90.2% of participants, respectively, and the vast majority (89% for trauma and 82.7% for taphonomy) thought explicit approval should be sought for such research.

While investigating current body donation practices in forensic anthropology, it was found that only 37% accepted donations from medical examiner/coroners and only 17.9% accepted unclaimed individuals. Responses regarding donation restrictions cite health status as the most common reason for donation rejection. Respondents indicate that donors are used in taphonomy (40%), trauma (32%), other transformative (36%) research, and to create or expand fragmentary skeletal collections (12%).

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### Body Donation, Forensic Collections, Forensic Procedure

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## A18 Modeling Grave Depressions Using Open-Source Light Detection And Ranging (LiDAR) Data at the Forensic Anthropology Research Facility

*Mariah Moe, MA\**, Texas State University, San Marcos, TX; *Shelby Garza, MA*, Texas State University, San Marcos, TX; *Nicholas Herrmann, PhD*, Texas State University, San Marcos, TX

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the utility of open-source Light Detection And Ranging (LiDAR) data for the detection of ground surface depressions consistent with single or mass graves and how hydrologic modeling techniques can be used in conjunction with mapping software to model these depressions.

**Impact Statement:** This presentation will impact the forensic science community by presenting an alternative method for grave detection that can be accomplished using free, open-source data.

The Forensic Anthropology Research Facility (FARF), located in Hays County, TX, is used for a variety of research projects through the Forensic Anthropology Center at Texas State's (FACTS) willied body donation program. This includes the burial of remains in single and mass graves, which provide the opportunity to investigate whether the ground surface depressions associated with these inhumations can be adequately and consistently detected using free, aerial-based LiDAR remote sensing data offered through the Texas Natural Resources Information System (TNRIS). No systematic evaluation of grave detection using aerial-based LiDAR at FARF has been conducted, yet recent studies have demonstrated moderate success in the detection of single and mass graves using terrestrial-based equipment.<sup>1</sup>

This study analyzed TNRIS LiDAR point cloud data at a 50cm resolution from 2017 to examine whether elevation changes consistent with known burials at FARF could be effectively detected using ground points parsed from the large datasets.<sup>2</sup> Geographic Information Systems software (ArcGIS Pro) and the LASTools package were used to generate topographic and slope maps. Additionally, digital elevation models were created to identify burial depressions.

An adaptation of hydrologic modeling techniques was employed in which digital elevation models typically used for watershed analysis and floodplain management were investigated for the presence of "sinks" (areas of low elevation for which all surrounding data cells represent higher elevation values). These areas of low elevation were noted to be consistent with the location of several known burials, including multiple single and one mass grave located at the facility.

The final model readily identified ground surface anomalies associated with large depressions resulting from long-term burials. This included depressions that contained human remains and ones in which the remains had previously been excavated, but the surface depression persisted. This study demonstrates the potential for open source LiDAR data to provide enough resolution to identify both single and mass graves. Additionally, these data have the potential to help direct on-the-ground investigative efforts in the search for clandestine burials. As more remote sensing data are collected and disseminated, future studies can evaluate the temporal and topographical changes associated with these additional datasets and whether smaller and/or more shallow inhumations can consistently be detected.

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### Grave Detection, LiDAR, Depression

## **A19 Discovering Clandestine Human Remains Using Unmanned Aerial System-Based Remote Sensing**

*Daniel Wescott, PhD\**, Texas State University, San Marcos, TX; *Gene Robinson*, Gene Robinson Consulting, Inc, Wimberley, TX; *Derek Anderson, PhD*, University of Missouri, Columbia, MO; *Shane Seitz*, Unmanned Systems Research, Inc, Saskatoon, Saskatchewan, Canada

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**WITHDRAWN**

## A20 A Robust Methodology for Stable Isotope Analysis of Human Tooth Enamel

Francesca Spencer, BS, BA, University of Utah, Salt Lake City, UT; Kirsten Verostick, PhD, University of Utah, Salt Lake City, UT; Alejandro Serna, PhD, University of Utah, Salt Lake City, UT; Lesley Chesson, MS, DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI; Gregory Berg, PhD, DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI; Gabriel Bowen PhD, University of Utah, Salt Lake City, UT

**Learning Overview:** After attending this presentation, attendees will have an improved understanding of how standardized sample preparation protocols can improve the comparability of stable isotope data produced in different laboratories and support more robust interpretations of stable isotopic data in human provenance investigations.

**Impact Statement:** This presentation will identify the most critical considerations for developing a robust, standardized methodology for the Stable Isotope Analysis (SIA) of human tooth enamel, which, if adopted, will support generations of data that can be replicated and is comparative across laboratories.

Traditional forensic analyses such as DNA and fingerprinting are often limited in the identification of human remains due to sample loss, contamination over time, and ability to locate reference samples. In the past three decades, SIA has become increasingly important in determining region of origin and life histories from unidentified remains. Forensic scientists and anthropologists have been guided by SIA in various cases, resulting in positive identifications.

Tooth enamel is a useful tissue when analyzing stable isotopes because its oxygen isotope ratios are resistant to change after the enamel has developed. It has been established that there is a relationship between the oxygen isotopes of enamel and those of water from the enamel's region of origin, providing a basis for inferring an individual's region of origin based on tooth enamel oxygen isotopes.<sup>1</sup> However, laboratories conducting isotope analyses for forensic purposes have not established a uniform methodology, leading to large interpretable differences and potential biases that limit comparison of results and interpretations between laboratories.<sup>2</sup>

This study builds on a history of methodological research on archeological samples and presents a series of experiments designed to test and quantify the effects of different sample preparation and analysis methodologies on the measured isotopic composition of modern human tooth enamel.<sup>3</sup> The experiments included treatments comparing different tooth enamel sample particle sizes, storage conditions during pretreatment and prior to Isotope Ratio Mass Spectroscopy (IRMS) analysis, organic contaminant removal treatments, and mass spectrometric analysis conditions. These experiments used relatively large sample sets and replicate analyses to support quantification of effect sizes.

Preliminary results from these experiments showed that the reproducibility of oxygen isotope measurements improved from 0.17‰ to 0.09‰ (1 standard deviation) if samples were ground to a fine and uniform particle size (125µm), likely due to improved homogenization of the samples. They also demonstrated a small (0.18‰) but significant offset ( $p < 0.1$ ) between the measured oxygen isotopic composition of fine- and coarse-grained samples. In the same experiment, the Carbon Dioxide (CO<sub>2</sub>) yield from the fine-grained samples was 28% higher than that for the coarse-grained samples. This is attributed to the observed isotopic effect to particle-size-dependent differences in reaction efficiency during mass spectrometric analysis. In contrast, the data show no detectible impact of variations in sample storage conditions (exposed to the ambient environment or stored in a desiccator). This suggests that oxygen exchange between atmospheric water vapor and enamel does not affect the isotopic values, even for finely ground samples.

The results of these experiments suggest “best practices” that can easily be adopted in SIA tooth enamel protocols to improve data comparability in inter- and intra-laboratory settings. In addition, they highlight methodological variables that have little impact on SIA results and need not be standardized. These methodological guidelines should help establish a firm methodological basis for building high-quality tooth enamel SIA reference databases and thus increase the applicability of SIA in forensic science for use in human identification efforts.

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### Stable Isotopes, Forensic Provenance, Methodology

## A21 Repatriation and Responsibility: Consideration Beyond Traditional Constructs

*Rose Leach, MA\*, Kent State University, Kent, OH; Kerianne Armelli, MS, Kent State University, Kent, Ohio, USA*

**Learning Overview:** Attendees can expect a comprehensive overview of repatriation practices, emphasizing the need to critically reexamine current collections housed at museums and universities beyond just the demands of the Native American Graves Protection and Repatriation Act (NAGPRA). Attendees will also learn about the multiple ethically sourced osteological collections they can utilize for current and future research, with particular consideration for digital databases.

**Impact Statement:** This presentation strives to bring continued awareness to the issues in repatriation by highlighting not only problems within the context of NAGPRA, but also providing additional insight into ethical considerations of skeletal remains and collections. This discussion will also provide resources on modern, ethically built collections that can be utilized for current and future research.

While forensic anthropology has recently begun to recognize a need for a culturally sensitive approach to training and research, many institutions actively make use of unrepatriated remains of Native Americans and individuals whose use within education and research today is ethically questionable (e.g., compared to individuals and groups whose skeletal donation was given explicit self- or next-of-kin consent). While there is a known responsibility to notify and return Native American remains through NAGPRA, these collections often continue to be used in educational settings. Due to the complex nature of relationships between faculty and trainees, along with the political and legal issues that accompany careers in the Academy, enacting change that leads to repatriation can be extraordinarily difficult.

The continued use of Native Americans and individuals whose acquisition was ethically questionable, or whose tribal affiliation warrants further investigation within collections in anthropology, has been argued as a necessity, citing limited resources for research and teaching as reason.<sup>1</sup> While the use of these collections can continue with explicit permission and communication with the ancestral communities of the descendants, the problem arises in the refusal to begin the process of seeking permission. Additionally, non-Native remains that may have been collected through unethical means, by current standards, are often overlooked, with many failing to question the ethics behind their acquisition. The use of historic and prehistoric samples in the development of forensic methods has been proven to be counterproductive, as current needs within the field require these methods to be modified for modern populations.<sup>2</sup>

This presentation seeks to bring awareness to the field regarding the active oversight of forensic anthropologists in the repatriation of Native American and other non-ethically sourced individuals/groups. With the advent of digital collections as well as physical ones comprised of modern individuals, it is clear that there are viable alternatives for research that can be utilized. Collections such as the William M. Bass, the New Mexico Decedent Image Database, and The Skeletal Series at Western Michigan University have developed collections consisting of consenting individual donors or next-of-kin donations, showing this can be done.

It is in the benefit of practicing forensic anthropologists to hold each other and their institutions accountable in the use of these collections and work toward more ethically sourced donations. This presentation will highlight the issues within modern anthropological skeletal collections and the resources and path forward to decolonizing collections.

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### Repatriation, Decolonization, Skeletal Collection Management

## A22 Morphometric Comparison of Eight Small Non-Human Species of Long Bones to Juvenile Human Long Bones for the Purpose of Identification in a Forensic Context

Kaitlyn Schoonover, BS\*, Mercyhurst University, Erie, PA; Luis Cabo MS, Mercyhurst University, Erie, PA

**Learning Overview:** Attendees to this presentation will gain a better understanding of the importance of non-human osteology in forensic contexts, as an essential tool to assess the forensic significance of skeletal remains based on human versus non-human determinations, particularly in scenarios involving potential adolescent remains.

**Impact Statement:** Human versus non-human determinations represent a significant portion of a forensic anthropologist's workload. Quick assessments of forensic significance based on this factor prevent significant waste of time and resources. Determinations based on positive species identification are much more robust and reliable, and less susceptible to error than those based simply on excluding human origin. In the training of forensic anthropologists, stress is often placed mainly on the identification of large species of mammals, in the body size range of human adults. However, the remains of small mammals can also be confused with those of subadults; with the added complication of small-sized species being more abundant and diverse in non-human communities.

While the identification of elements from immature vertebrates requires more thorough examinations and a stronger osteology background, the remains of adult small vertebrates are also often mistaken for potential human bones as a result of the common misconception among the public that bones of infants and juveniles are just smaller versions of fully developed adult bones. Any system that could be used to streamline those identifications at the initial stages of the investigation would significantly reduce the waste of investigative time and resources.

However, there are few studies using morphometrics specifically to compare human and non-human bones, let alone any studies using juvenile human bones in comparison to various small mammal species. A 2016 study tried to determine whether bone morphometry analyses on long bones would be robust enough for this purpose, obtaining promising results.<sup>1</sup> Nonetheless, those authors utilized ratio transformations in their analyses, a strategy that has been proven to be ineffective and highly problematic in morphometry.<sup>2</sup>

A pilot study is presented here illustrating two alternative strategies to streamline the identification of small vertebrate elements that are frequently mistaken as potential infant human remains. One strategy utilizes morphometric analysis, with the second being the classic dichotomous key structure.

Although mammals, along with other vertebrates, have homologous skeletal structures, their function and biomechanics varies across taxonomic groups, leading to easily interpreted morphological differences between taxa; for example, the differences in the use of arms/forelimbs in bipedal (grasping or flying) versus quadrupedal animals (walking and/or running). This difference in biomechanical function leads to differing structure morphologies that can often be observed as easily recognizable discriminant traits.<sup>3</sup> Dichotomous keys based on the visual assessment of these traits are widely utilized in zoology to identify species based on these traits.

However, morphology also changes with age, with skeletal elements of immature individuals being more similar across species than their adult counterparts. The morphometric approach has the advantage of detecting and encapsulating not only size and raw shape differences, but also allometry. This allows us to extend the results to a wider range of ages and body sizes and ages, by factoring in allometric patterns in addition to anatomical differences related to function and biomechanics. The biometric model is intended to take advantage of this factor, also presenting some advantages for easier automation through software applications.

The study sample was obtained from the Mercyhurst University zooarchaeological comparative collection. A set of small vertebrate species was selected based on their high frequency in North American environments, as well as on their potential to be mistaken for human infants or fetuses based on their body size. Skeletal elements were 3D scanned with an Artec Space Spider. Scans were also utilized to create dichotomous keys. All statistical analyses were carried out using GraphPad Prism 9.2.0. It was determined that lower limb elements are better than the upper limb when measuring morphometric differences between non-human and fetal human bones, with the femur showing the most robust results. Although this is only a preliminary study, the statistical data show that there are significant differences between non-human and juvenile human bones, depending on the bone and features analyzed.

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### Forensic Anthropology, Zooarchaeology, Morphometrics



## A23 A Spatiotemporal Analysis of the National Missing and Unidentified Persons System (NamUs) Unidentified Person Cases along the United States-Mexico Border

Justin Z. Goldstein, MA\*, Texas State University, San Marcos, TX; Courtney Siegert, MA, Texas State University, San Marcos, TX; Nicholas Herrmann, PhD, Texas State University, San Marcos, TX

**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the spatial distribution of National Missing and Unidentified Persons System (NamUs) Unidentified Person (UP) cases along the United States-Mexico Border and how these cases relate to deaths reported by the Border Patrol.

**Impact Statement:** This presentation will impact the forensic science community by providing important context for understanding changes in reported deaths across the United States-Mexico Border over time.

Unidentified human remains present a significant challenge for forensic practitioners in the United States, particularly given its decentralized medicolegal framework.<sup>1</sup> Identification efforts on the United States national scale must navigate the various protocols, financial resources, and organizational barriers that are unique to each state and/or county. NamUs acts as a powerful tool for connecting forensic professionals and investigating agencies across the United States, although engagement across the nation remains inconsistent.<sup>2,3</sup> However, along the United States-Mexico border, where thousands of migrants are apprehended or found deceased, NamUs has played a crucial role in identification efforts.<sup>4,5</sup> Thus, understanding the relationships between NamUs UP cases and Border Patrol statistics may provide important insights into the factors that have contributed to death along the border over time. This study sought to explore these relationships, asking: (1) can NamUs UP cases, apprehensions at the border, and Border Patrol staffing be used to accurately predict reported deaths across Border Patrol sectors, and (2) do the relationships between these variables change over time?

NamUs UP cases from Arizona, California, Nevada, New Mexico, Oklahoma, and Texas ( $n=6,857$ ) were exported from NamUs 2.0 and geocoded in ArcGIS<sup>®</sup> Pro 2.8 to county shapefiles obtained from the United States Census Bureau.<sup>6,7</sup> Border Patrol sector reports on staffing, apprehensions, and deaths were obtained from United States Customs and Border Protections.<sup>8</sup> In ArcGIS<sup>®</sup>, Border Patrol sector statistics and NamUs UP cases were joined to Border Patrol sector shapefiles, which were generated using publicly available jurisdictional data.<sup>9</sup> In each sector, UP Cases and Border Patrol statistics were summarized for three approximately equal time periods: 1998-2006, 2007-2013, and 2014-2019. Ordinary Least Squares (OLS) regressions were performed in ArcGIS<sup>®</sup> Pro for each time period, with reported deaths as the dependent variable. In addition, Border Patrol apprehensions per sector by migrant region of origin were summarized by year to contextualize regression results over time.

OLS regression models indicate no consistent predictors for reported deaths over time. The lack of significance between NamUs UP cases and reported deaths is likely due not only to the inconsistent application of NamUs across Border Patrol sectors, but also to the unknown number of previously identified cases that are no longer listed in NamUs 2.0. Moreover, Global Positioning System (GPS) location data for NamUs cases is inconsistently collected, impacting the precision of spatial analyses. Removing NamUs UP cases from OLS regression models shows a more consistent relationship between border apprehensions and reported deaths ( $p < 0.01$ ). Additionally, adjusted R-squared values decreased from 0.82 to 0.57 between the 2007-2013 and 2014-2019 models. Considering that Texas surpassed Arizona in migrant deaths in 2012, lower model performance may reflect changing migration trajectories and demographics, the decentralized medicolegal system, and the difficulties recovery contexts encountered in Texas.<sup>5</sup>

Although NamUs UPs are not strong predictors of reported deaths along the United States-Mexico border, data here likely represents an incomplete account. Including higher-resolution NamUs data, particularly GPS location and data from previously resolved cases, may improve model precision.

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### United States-Mexico Border, Unidentified Persons, Migration

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## A24 Developing a Tissue-Specific Multi-Isoscape ( $\delta^{18}\text{O}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ) to Estimate Region of Origin

Julianne Sarancho, MS\*, Arizona State University, Tempe, AZ; Alexis Baide, MA, University of Utah, Salt Lake City, UT; Clare Dittmore, BA, Montana State University, Bozeman, MT; Thomas Delgado, BA, California State University-Chico, Chico, CA; Gabriel Bowen, PhD, University of Utah, Salt Lake City, UT

**Learning Overview:** After attending this presentation, attendees will understand the utility of creating multi-isotope tissue-specific isoscapes to estimate region of origin via Stable Isotope Analysis (SIA).

**Impact Statement:** This presentation will impact the community by describing a novel method of combining isotope systems in the creation of tissue-specific isoscapes for region of origin estimation to increase the accuracy of stable isotope analytical methods.

Forensic applications of SIA have shown this method's utility to aid in the identification of unknown remains. As its use becomes more widespread, methods to improve the ability of isotopic analysis to provide geographic history of unidentified human remains are essential. Currently, many forensic applications of SIA rely on the comparison of isotopic values from human tissues (i.e., bone, teeth, or hair) to predicted environmental isotopic values derived by modeling precipitation, tap water, and/or bedrock maps for particular regions, commonly referred to as isoscapes.<sup>1-3</sup> While this has shown to be a useful comparison, it cannot be assumed that environmental isotopic values are equivalent to the isotopic signatures preserved in the human body because this method fails to account for tissue-specific metabolic processes which may cause isotopic fractionation. To remedy this assumption, it is recommended that environmental isoscapes are calibrated with human tissues using known matrix-matched samples.

To assess the efficacy of a human tissue-specific isoscapes, this study developed a multi-isotope model of  $d^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  from hair as a function of tap water to infer the region of origin for individuals. For  $^{87}\text{Sr}/^{86}\text{Sr}$ , a linear regression was generated using data from Ammer et al. that consists of hair and associated tap water samples collected in Mexico.<sup>4</sup> The linear regression was used to calibrate an environmental  $^{87}\text{Sr}/^{86}\text{Sr}$  isoscape of the United States. A  $d^{18}\text{O}$  isoscape was constructed using hair data from Ehleringer et al. and existing tap water isoscapes for the United States.<sup>5</sup> The  $d^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  isoscapes were then stacked to provide a multi-isotope model. All modeling, mapping, and statistical analyses were conducted in R version 3.6.2 using the ASSIGNR package.<sup>6</sup>

To test the accuracy of the multi-isotope model,  $d^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  of hair samples with known region of origin (obtained from Tipple et al.) were evaluated.<sup>7</sup> The model provided estimates that included the correct region of origin for 91% of the known samples tested.

The high accuracy of the multi-isoscape model highlights the utility of multi-isotope tissue-specific models in forensic applications. Despite this high accuracy, considerations to ensure consistent data reference scales and the ways in which local  $^{87}\text{Sr}/^{86}\text{Sr}$  values may contaminate a sample in question must be taken into account. This study serves as an initial multi-isotope model for human hair and may serve as an example for the development of additional models.

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### Stable Isotope Analysis, Multi-Isotope Model, Hair Isoscape

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## A25 Last Seen Location: Using Missing Persons Data for Identification in Forensic Anthropology.

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**Learning Overview:** After attending this presentation, attendees will understand how the spatial precision of a missing person's last-seen location relates to their case's closure and how the value of spatial data relate to the identification of unidentified individuals.

**Impact Statement:** This presentation will impact the forensic science community by expanding the scope of the types of data that forensic anthropologists use as it relates to current forensic casework.

The study and practice of forensic anthropology is centered on unidentified individuals and, as practitioners, improving the field's ability to successfully assist in identification efforts. Traditional methodological practice relies on “the big four”—age, ancestry, sex, and stature—to create a biological profile. In recent years, there has been an increasing call by forensic anthropologists to expand beyond skeletal material in their analysis and interpretation of unidentified remains to include sociocultural data.<sup>1</sup>

With the augmentation from the sociocultural data, the biocultural profile provides a more holistic understanding of an unidentified individual and may provide a more effective means to establish possible identifications. Furthermore, the inclusion of an unidentified individual's geographic recovery location has been shown to be an effective biocultural identifier in establishing a putative identification by cross-referencing missing persons cases.<sup>2</sup> However, the spatial relationship of establishing a putative identification involving recovery location is dependent on the missing person's case report and potentially the degree of knowledge of an individual's last-seen location.

To explore the relationship between an individual's last-seen location and case outcomes, this study analyzed a total of 419 open and closed missing persons reports from the Louisiana Repository for Unidentified and Missing Persons Information Program of individuals last seen in the past 64 years from 1956–2020.<sup>3</sup> Missing persons reports were categorized by the level of spatial precision available (e.g., exact address, street, city, parish/county, or unknown). The data indicate that there is no significant relationship between the level of spatial precision and overall case closure rate. However, temporal data, when available, suggests a correlation between the level of spatial precision and average time from when the individual was last seen to their case's closure; missing person cases with spatial precision at the parish level remain open for an average of 7,650 more days than cases with an exact last-seen location. This present research shows the utility of spatial data in forensic investigative efforts and highlights the varied relationship spatial information has to unidentified persons cases in forensic anthropology.

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### Missing Persons, GIS, Identification

## A26 Understanding the Spatial Relationships Between NamUs Unidentified and Missing Persons Cases in Texas

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the spatial relationship between National Missing and Unidentified Persons System (NamUs) Unidentified Persons (UP) and Missing Persons (MP) cases in Texas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contextualizing spatial trends in NamUs cases in relation to the demographic variation in Texas.

Existing literature has contextualized unidentified human remains and MP as vulnerable populations, subject to larger systems of structural violence in the United States.<sup>1</sup> According to NamUs, as of August 2021, there are 1,651 MP and 1,737 UP in the state of Texas.<sup>2</sup> However, given that the medicolegal systems in Texas are decentralized across 254 counties, it is likely that MP and UP cases have differential access to investigative resources.

The purpose of this study is to explore the similarities and differences between NamUs UP, NamUs MP, and county demographics to answer the following questions: (1) is there non-random spatial distribution in the NamUs UP and MP cases in Texas, and (2) if the spatial relationships are non-random, are there similar spatial patterns in county demographics?

Data were collected on population, self-reported race, self-reported ethnicity, average income, and poverty rate for each county in Texas from the United States Census 2019 ACS 5-Year Survey.<sup>3</sup> MP and UP data were collected from NamUs on August 28, 2021. Maps were generated to visually represent the UP, MP, and county demographic data in ArcGIS® Pro using the symbology feature. Global Moran's I spatial autocorrelations were run to test for non-random spatial distributions of MP cases, UP cases, and county demographics.

The results of the Global Moran's I spatial autocorrelation test showed that significant clustering is occurring with the distribution of MP by population ( $p < 0.01$ ;  $z = 7.66$ ), UP by population ( $p < 0.01$ ;  $z = 7.50$ ), Hispanic individuals by population ( $p < 0.01$ ;  $z = 23.61$ ), and Black individuals by population ( $p < 0.01$ ;  $z = 19.37$ ). Furthermore, the clustering pattern reveals that the areas with the higher number of UP and MP cases are also the areas with the highest number of Hispanic and Black individuals by population.

These results show that, when standardized by population size, there is a statistically significant correlation between the distribution of Black and Hispanic individuals in Texas and the distribution of unidentified and missing persons in Texas. Collectively, these results may speak to the impacts of structural violence on socially marginalized communities, defined here as avoidable limitations placed on groups of people by society that prevents them from achieving an otherwise attainable quality of life.<sup>4</sup> This project serves as an important step in understanding how structural violence can impact individuals both during life and death.

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### Forensic Anthropology, Spatial Analyses, Structural Violence

## A27 Initial Review of the Long-Term Unidentified Cases Managed by the Forensic Anthropology Center at Texas State University

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the contextual and demographic information currently available for long-term unidentified cases at the Forensic Anthropology Center at Texas State University (FACTS).

**Impact Statement:** This presentation will impact the forensic science community by presenting data on a representative group of forensic cases managed by FACTS to highlight common discrepancies in long-term unidentified casework at academic institutions.

When medicolegal agencies and jurisdictional authorities request forensic anthropological consultation on unidentified human remains, forensic anthropologists in academic institutional settings are often responsible for the continued curation of unidentified human remains after analysis is complete.<sup>1</sup> Unidentified human remains represent a marginalized group of individuals in the global humanitarian crisis of the missing and unidentified, and academic institutions face challenges in balancing both consultation and stewardship when managing such vulnerable individuals.<sup>2</sup> For cases that remain unidentified after traditional forensic methods are applied and reported, the challenges become multifold as long-term storage inherently requires casework reassessment, further procedural standardization, and additional detailed recordkeeping over time.

The Identification & Repatriation Initiative (IRI) was created at the FACTS in 2021 to standardize the inventory of and re-engage identification efforts for all unidentified individuals retained in long-term storage at FACTS, particularly for cases analyzed prior to the formation of FACTS in 2008. Dates of discovery for these individuals span from the 1960s to the present. This presentation summarizes the IRI's initial findings, focusing on the availability of associated contextual data and the completeness of forensic analyses already conducted. The IRI also surveyed the demographic breakdown of long-term unidentified cases in order to better understand the communities represented by these individuals.

The IRI reviewed all existing cases and associated documents and found that 95% of the total cases required additional analysis or reanalysis. Of these, 80% were deemed to be modern and therefore medicolegally significant. Cases that demonstrated evidence of identification attempts beyond submission of the biological profile included: (1) bone samples apparently removed for DNA submission (24%); (2) documentation of submission of bone samples for DNA profiling by the University of North Texas Center for Human Identification (16%); and (3) entry into the National Missing and Unidentified Persons System (NamUs) (11%). Perhaps most notable, only 32% of all forensically relevant cases had an associated and completed forensic anthropological report. Of those with estimated age, sex, and population affinity information recorded, the majority were estimated as male (62%), between the ages of 15–50 (82%), and of Hispanic (33%), American White (30%), or American Black (4%) affinity, with the rest being of unknown or undetermined affinity. These initial estimations, paired with the associated reports of discovery from counties along the United States-Mexico border, suggest many of these individuals may have suffered from broader vectors of structural inequality and vulnerability during life.<sup>3</sup> Moreover, as they remain unidentified throughout the years, those lived experiences are reflected in their marginalization in death.<sup>4</sup>

Although the problems that the IRI encountered when reassessing the institutional holdings of the unidentified dead were significant, the IRI is working toward new directions in identification for these individuals. The IRI is hopeful that reporting this general information on the unsolved forensic cases currently housed at FACTS will promote conversation between academic institutions regarding the stewardship, management, and solvability of long-term unidentified cases. By highlighting these discrepancies and contextualizing these decedents as embedded in larger systems of structural violence and vulnerability, this presentation encourages a collective discussion of potential solutions for identification barriers and institutional challenges.

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### Unidentified Human Remains, Long-Term Dead, Academic Institutions

## A28 Stature Estimation From Facial Parameters—A Computed Tomography (CT) Scan-Based Observational Study in a South Indian Adult Population

*Siddhartha Das, MD\*, Forensic Medicine & Toxicology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry INDIA*

**Learning Overview:** After attending this presentation, attendees will be able to appreciate the role of CT measurements of facial parameters for estimating the stature in a south Indian population.

**Impact Statement:** This presentation will impact the forensic science community by shedding light on the importance of CT measurements of facial parameters for estimating the stature in a south Indian population.

**Background:** Establishing the identity of a deceased individual is vital in forensic casework. Stature estimation helps in partial identification of the individual and is determined by anatomical or mathematical methods. The anatomical method is applied when the entire skeleton is available for examination; however, in many cases, mutilated or dismembered bodies are found and only the mathematical method can be applied. In some cases, only the cranium may be available for forensic evaluation. Researchers who have evaluated stature from the cranium have used mostly living individuals or the dry bone.<sup>1-3</sup> In recent years, CT measurements of the cranium have been used for estimating stature. The objective of this study was to estimate stature from CT scan measurements of facial parameters in a south Indian adult population.

**Methods:** A total of 330 males and 181 females, ranging in age from 21-60 years, scheduled for elective CT of the head and neck were recruited for the study. The patient's height was measured before obtaining the CT scan. Bizygomatic Breadth (BZB), Minimum Frontal Breadth (MnFB), Nasal Breadth (NB), Nasal Height (NH), Orbital Breadth (OB), Biorbital Breadth (BB), Interorbital Breadth (IOB), Orbital Height (OH), and Upper Facial Height (UFH) parameters were measured from reconstructed CT images. Univariate and multivariate linear regression was performed to establish a predictive stature estimation model using these parameters for a south Indian population.

**Results:** The mean stature for males was 167±6.03cm and 157.40±6.33cm for females. All the studied parameters showed statistically significant positive correlation with stature. Multiple linear regression analysis using backward elimination containing all the independent predictors revealed a statistically significant model, with a  $r^2$  value of 0.17. There was no violation in model assumptions, such as linearity, constant variance, or residual distribution. Multicollinearity was ruled out using the variance inflation factor. When correlation was studied individually in the female and male sample, only NH ( $p=0.02$ ), OBL ( $p=0.003$ ), OHR ( $p=0.03$ ) in the female sample and BZB ( $p<0.001$ ) NH ( $p<0.001$ ) and UFH ( $<0.001$ ) in the male sample demonstrated a statistically significant positive correlation with stature. The multivariate linear regression predictive model using backward elimination of all independent variables yielded a statistically significant model with an  $r^2$  value of 0.104 with predictors BZB, NH, and LOH for males and an  $r^2$  value of 0.06 with predictors NH and LOB for females.

**Conclusion:** This CT-based observational study demonstrated that anterior facial parameters are positively correlated with stature, but the predictive accuracy for estimating stature in a south Indian adult population is low.

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### Stature Estimation, Facial Parameters, South Indian Population

## A29 OsteoFlo: A FIJI/ImageJ Toolkit for Semi-Automated Identification and Characterization of Fluorescently Labeled Secondary Osteons

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**Learning Overview:** The goal of this presentation is to introduce an open source FIJI/ImageJ (NIH) toolkit for morphometric analysis of vascular pores and secondary osteons on histological images of cortical bone tissue.

**Impact Statement:** This presentation will impact the forensic science community by introducing novel software that significantly expedites histomorphometric analysis using automation. Vascular pores and secondary osteons are typically manually identified on microscopic images, with forensic application of their morphometry to assess histological age-at-death, remodeling activity, and bone tissue quality.

The developmental dataset was femora of New Zealand White rabbits, who were subcutaneously injected with a bone-labeling fluorochrome, calcein, at three timepoints over eight weeks. This toolkit identifies vascular pores on Differential Interference Contrast (DIC) microscopic images, stemming from previous work on brightfield images.<sup>1</sup> Secondary osteons formed during this period are calcein-labeled, as visualized with fluorescent microscopic images. Toolkit macros are fully automated unless denoted with an (M).

### Toolkit Macros for Pore Analysis on DIC Images:

**Pre-Processing:** The DIC image is evenly illuminated through application of a high pass filter, contrast enhancement, and background subtraction.

**Pore Extractor:** Probable pore Regions Of Interest (ROIs) are extracted from the DIC image using an Intermodes auto-threshold, particle size and circularity thresholding, and binary closing.

**Pore Modifier (M):** Pore ROIs are manually edited using custom keyboard shortcuts, including auto-saving.

**Pore Analyzer:** Pore ROIs are classified as cortical or trabecularized based on proximity to the marrow cavity versus minimum diameter.<sup>2</sup> Pores are also regionally subdivided between anatomical quadrants (long bones) or cutaneous and pleural halves (ribs), calculated using the centroid and major axis.<sup>3</sup> Summary pore morphometry is calculated for each pore type within each region, including percent porosity, pore density, and mean pore size and shape descriptors.

### Toolkit Macros for Osteon Analysis on Fluorescent Images:

**Osteon Extraction:** Probable osteon border ROIs are extracted using local contrast enhancement, histogram equalization, an Intermodes auto-threshold, and binary opening. Fluorescent rings within osteons representing calcein labels are extracted as a binary image using background subtraction and an auto local Phansalkar threshold.

**Osteon Border Correction (M):** Osteon border ROIs are manually edited using custom keyboard shortcuts, including osteon splitting and expansion.

**Osteon Type:** Osteon border ROIs are regionally assigned as in Pore Analyzer. An ellipse is fitted over each ROI, and its major axis is superimposed on the binary image of its calcein labels. An intensity profile along this major axis displays peaks that correspond to the number and spacing of calcein label intersection. This intensity profile is used to guess Osteon type (forming, single, double, or triple labeled), Mineral Apposition Rate (On.Mar), which is the distance between two consecutive calcein labels, and osteon Wall Thickness (W.Th), which is the distance from the pore centroid to the osteon border.<sup>4</sup>

**Osteon Type Correction (M):** Osteon border ROIs are colored by guessed osteon type, and coordinates for guessed On.Mar spacing and W.Th pore centroids are superimposed as overlays. Custom keyboard shortcuts quickly change osteon type and re-locate coordinates for On.Mar and W.Th. Summary morphometry is automatically calculated for each region, including osteon type counts, corrected On.Mar and W.Th, and mean size and shape descriptors.

**Mineralizing Surface Per Bone Surface:** The ratio of labeled to unlabeled bone on the periosteal (Ps.MS/BS) and endosteal (Es.MS/BS) surfaces is calculated as the number of white pixels intersected by the bone surface perimeter.<sup>4</sup>

**Resorption Cavities (M):** Probable unlabeled resorption cavities are identified as pore ROIs that do not intersect with osteon borders and fall above the lowest histogram bin value for pore size. Users can manually change this classification.

Image processing automation improves user consistency while significantly reducing time investment for histomorphometric analysis.

*Certain work included here is supported by the National Institute of Justice, Award 2018-DU-BX-0188. The opinions, findings, and conclusions expressed are those of the authors and do not necessarily reflect those of the Department of Justice.*

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**Image Processing, Histomorphometry, ImageJ**



## A30 A Multi-Isotopic Analysis of Ajnala Skeletal Remains for Provenance of Their Local or Non-Local Origin, Geographic Affinity, and Mobility Patterns

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**Learning Overview:** After attending this presentation, attendees will get sensitized about the importance of stable isotope analyses in provenance of unknown human remains recovered from forensic anthropological contexts.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that the chemical markers of identity imprinted in human teeth and bones as stable isotopes can help in identification of origin, geographical affinity, and movement history (if any) of the badly damaged and commingled human osseous remains

The technique of stable isotope analysis is still underutilized in modern forensic casework, probably due to its novelty, ongoing research, additional expenses involved, the unavailability of databases for different geographic regions of the world, and, lastly, the expertise of the investigator. In the present study, the dentine collagen and enamel samples collected from randomly selected third mandibular molars were subjected to detailed multi-isotopic analysis ( $\delta^{13}\text{C}$ ,  $\delta^{14}\text{N}$ ,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) using Isotope Ratio Mass Spectrometer (IRMS) with the objective of narrowing down the potential region of origin (local or non-local to site), geographic affiliation, and mobility history of unknown human remains exhumed non-scientifically from an abandoned well at Ajnala, India. The mandibular third molars were considered for analysis to avoid duplication of individuals as the remains were commingled and badly fragmented. It was hypothesized that Ajnala individuals belonged to a specific regiment, having recruited soldiers from a particular geographic region who were non-local to the site. The standard protocols were followed for the pre-treatment of the tooth dentin and enamel samples for isotope analyses. The collagen from the vialled dentine samples was extracted using Merck's Amicon® Ultra 1.5 micro-filter columns before their encapsulation was done for sending them for  $\delta^{13}\text{C}$  and  $\delta^{14}\text{N}$  analyses.

The  $\delta^{13}\text{C}$  and  $\delta^{14}\text{N}$  values ( $N=21$ ) ranged from  $-18.7\text{‰}$  to  $-22.9\text{‰}$  (avg.  $-20.5\pm 1.2\text{‰}$ ) and  $+7.6\text{‰}$  to  $+11.7\text{‰}$  (avg.  $+9.3\pm 1.1\text{‰}$ ), respectively having C/N ratio varying from 2.8-3.6 (avg.  $3.4\pm 0.2$ ). The carbon, oxygen, and nitrogen isotope values of bones and teeth often represent the dietary as well as provincial signatures of the individuals.<sup>1</sup> Using Schoeninger et al., isotopic model of dietary reconstruction from  $\delta^{13}\text{C}$  and  $\delta^{14}\text{N}$  concentrations of collagen samples, it was found that Ajnala victims consumed mixed diets derived from both C3 and C4 resources.<sup>2</sup> Thus, the majority of the Ajnala individuals were consuming a varied diet of foods like wheat, rice, pulses, millet, maize, etc., and possibly animals foddered/grazed on C4 plants. These observations are in full agreement with the hypothesis that the human remains excavated from the site belonged to the reported regiment of a colonial army whose sepoy were killed and buried at the site following their mutiny during the 1857 rebellion.<sup>3</sup> A particular division of the military would be expected to draw from different members of the public and would not be expected to be a uniform group of individuals.

The projected source water  $\delta^{18}\text{O}$  v. VSMOW values for the majority of samples ( $n=32$ ) varied from  $-5.89\text{‰}$  to  $-9.00\text{‰}$  (avg.  $-7.64\pm 0.03\text{‰}$ ), and these results were consistent with Orissa, West Bengal, Bihar, parts of Meghalaya, and parts of Manipur as potential regions of geographic origin.<sup>4,5</sup> The higher  $\delta^{18}\text{O}$  v. VSMOW values ranging between  $-9.31\text{‰}$  and  $-11.40\text{‰}$  (avg.  $-10.29\pm 0.13\text{‰}$ ) were consistent with Jharkhand as the potential region of geographic affinity of Ajnala skeletal remains, though it cannot be ruled out that these individuals came from a region or regions where riparian water fed by snow melt was the predominant source of potable water, thus suggesting the northern region of Assam along the Brahmaputra as their possible geographic affinity. The two samples having  $\delta^{18}\text{O}$  v. VSMOW values as  $-4.78\pm 0.06\text{‰}$  and  $5.33\pm 0.03\text{‰}$  reflected Tripura, southern Assam, and parts of Meghalaya or Manipur as their possible place of origin.

The  $^{87}\text{Sr}/^{86}\text{Sr}$  values of enamel samples ( $n=47$ ) varied from 0.727949 to 0.715938 (with an average of  $0.722463 \pm 0.00030$ ), which revealed that the less radiogenic ( $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.716$ ) values probably came from the individuals inhabiting the Ganga river basin, whereas the individuals with relatively higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ( $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.7200$ ) values probably resided in the Bihar and West Bengal states.<sup>6,7</sup> The 87/86Sr dataset revealed that the individuals analyzed were dependent on vastly different base geologies during their adulthood (since only mandibular third molars were analyzed). These observations are also fully congruent with the isotopic characterization one can expect for an infantry division comprising of a diverse set of people from different parts of the former British India. Each data point was compared with 87/86Sr data of the reported base geologies to ascertain the range of states/provinces to which the slain sepoy of the ill-fated battalion belonged. The  $^{87}\text{Sr}/^{86}\text{Sr}$  didn't support the local origin theory of Ajnala skeletal remains and attributed their affiliations to the Indian states of Uttar Pradesh, Bihar, and West Bengal.

This multi-isotopic dataset is the richest one for human skeletal remains excavated from a specific site in India and provided a scientific basis to the written accounts that Ajnala skeletal remains belonged to victims of the provinces of Awadh (northeastern Uttar Pradesh), Bengal, Bihar, and some northeastern Indian states. Thus, the results demonstrate the success of applying multidisciplinary approaches for forensic anthropological identification of unknown human remains. The detailed results will be presented in the textual, graphical, tabular, and diagrammatic forms in this presentation.

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### Ajnala Skeletal Remains, Multi-Isotope Analysis, Provenance

## A31 The Impact of British Biometry and Race Studies on the Origins of Forensic Anthropology in the United States

*Nicholas Passalacqua, PhD\*, Western Carolina University, Cullowhee, NC; Iris Clever, PhD, University of Chicago, Chicago, IL*

**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the origins of modern forensic anthropology as stemming from early biometric race scientists in Britain.

**Impact Statement:** This presentation will impact the forensic science community by providing new insights into the early development of forensic anthropology in the United States by connecting it to novel anthropological-biometric developments in early 20th-century Britain, associated with early biometric studies of race. Specifically, it will show how T. Wingate Todd and Wilton M. Krogman adapted methods from statistician Karl Pearson and anthropologists Sir Arthur Keith and Miriam L. Tildesley, putting them to new use in the United States and laying the foundations of modern American forensic anthropology.

Traditional histories of the emergence of forensic anthropology focused on the United States are typically separated by early forensic anthropology case consultation and early forensic anthropological scholarship in the late 19th century. Early casework examples include the murders of George Parkman in 1849 and Louisa Luetgert in 1897, in which both subsequent trials involved expert testimony on skeletal remains. Early scholarly contributions began with the 1878 work of Thomas Dwight, earning him the moniker of “father of forensic anthropology” by T. Dale Stewart.

It was not until Krogman’s 1939 publication, *A Guide to the Identification of Human Skeletal Material in the Federal Bureau of Investigation (FBI) Bulletin* that the discipline of forensic anthropology began to be formalized. Interestingly, little has been written about the impetus for Krogman’s FBI Bulletin paper. This project delves into the forensic anthropology-adjacent work being performed both in the United Kingdom and the United States in the 1920s and 1930s, leading up to, and directly shaping, Krogman’s landmark 1939 publication. This interdisciplinary presentation combines historical-archival research with citation mining and demonstrates key connections between American and British actors through historical correspondence, scholarly citations, and associated discussions.

The results demonstrate that anthropologist Miriam L. Tildesley, her former professor statistician, Karl Pearson, and her then-supervisor, renowned anthropologist Sir Arthur Keith engaged in efforts to “authenticate” (i.e., identify) historic crania in the 1920s and 1930s in the United Kingdom using new anthropological-biometric methods developed by Pearson. The work of Tildesley in particular served as inspiration for similar work in the United States by T. Wingate Todd. Contemporaneously, Krogman received a fellowship under Todd at Western Reserve University from 1928–29; and Todd introduced Krogman to Pearson and Keith. Krogman then traveled to the United Kingdom to work with Keith and Pearson from 1930–1931, before returning to Western Reserve University to work with Todd in his Anatomical Laboratory, until Todd’s passing in 1938. Additionally, while Todd’s casework is not well recorded, there is documentation of his examination of the remains of multiple individuals associated with the Cleveland Torso Murders that occurred from 1935–1938, at least one of which Krogman assisted with.

Based on these findings, while Krogman is credited for an early popularization of forensic anthropology, this would not have been possible without his mentorship by Todd and their interactions with Tildesley, Pearson, and Keith. First, this study argues that Todd treated Krogman as a protégé and to some degree supervised the production of Krogman’s 1939 publication *A Guide to the Identification of Human Skeletal Material in the FBI Bulletin*. Second, it is argued that both Todd and Krogman benefited from scholarly and personal discussions with Tildesley, Pearson, and Keith regarding what would now be considered forensic anthropological pursuits, and that these racial-biometric British methods shaped Krogman’s 1939 publication.

### History, Biometry, Wilton M. Krogman

## A32 Fifty Years in the American Academy of Forensic Sciences (AAFS)

*John Williams PhD\*, Western Carolina University, Cullowhee, NC; Katherine Kolpan, PhD, University of Idaho, Moscow, ID*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how the membership of the Anthropology Section of the AAFS has grown and changed over time.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with information regarding trends in Anthropology Section membership over time, information that underscores how the section has grown and diversified since its inception in 1972.

In 1972, 14 anthropologists attended the AAFS annual meeting as members of the newly created Physical Anthropology Section. In the 50 years that followed the inception of the Anthropology Section, membership has grown to over 600.

The AAFS has maintained section membership data for every year since 1972. These data, albeit limited in scope, serve as the basis for a demographic and membership study of how the Anthropology Section has progressed over its five decades. For the purposes of this study, members were designated only using standard cis-orientation. This assignment was based on first names and personal knowledge. Names that could not be given a standard cis-assignment were not included. Also tracked for each of the years was regular membership (Associate, Full, Fellow, Retired), Trainee Affiliate, and Student.

The 1970s, not surprisingly, were a period of slow steady growth for the section. Membership more than doubled. Although the number of women in the section more than doubled, the ratio of males to females was 4.4 to 1 at the end of the decade.

The 1980s was a decade of change for the section. The first Trainee Affiliates and the first Students were elected, and, by the end of the decade, these members would account for 24% of the section. At the beginning of the decade, the male:female ratio was 3.6:1, but by the end of the decade, the overall ratio would drop to 1.6:1, although among regular members, the proportion of females increased more slowly with an ending sex ratio of 3.2:1. The high proportion of women among Students and Trainee Affiliates was an indicator of the section's future.

Driven by a continued increase in Student membership throughout the 1990s, membership in the section almost doubled. By 1999, Students comprised nearly 1 in 3 section members. This changed the demographics of the Anthropology Section and by 1998, women outnumbered men. However, women had not yet reached equity in regular membership.

Membership steadily increased in the 2000s, but not as rapidly. Student membership dipped in the early part of the decade but rebounded to 35.7% of all members by 2009. Female members increased substantially. Student members accounted for over half (56.6%) of the total number of female section members. At the end of the decade, female regular members exceeded males for the first time.

As the section finished the most recent of its five decades, membership nearly doubled from 384 in 2010 to 604 in 2021. The biggest gains were among women, who now comprise 72.6% of all members in the Anthropology Section. During this time, the number of men in all membership categories remained relatively unchanged. Student membership fluctuated wildly, dropping in 2018 to only 128 from a high of 224 five years earlier.

There is also a relationship between Trainee and Student Affiliate designations. Prior to student membership, Trainee Affiliates were greater in numbers. In 1984, they made up 31.7% of the section's members, but decreased once Student membership became available. In 1995, the section had only three Trainee Affiliates. However, the past few years have shown a resurgence in Trainee Affiliate membership with 32 members in 2021.

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### Anthropology, History, Members

## A33 Pioneers of Color: Diversifying the Pantheon of Forensic Anthropology Luminaries

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**Learning Overview:** After attending this presentation, attendees will appreciate the lack of diversity among the most widely taught historical figures in forensic anthropology's foundations and learn of people of color who were also foundational to the discipline.

**Impact Statement:** The goal of this presentation is to impact the forensic science community by colorizing the pantheon of forensic anthropology's predominantly White forbearers in order to provide a more inclusive and diverse discipline.

The history of forensic anthropology in the United States centers specifically around the work of several key figures, particularly in how their research on human skeletal variation and collaboration with law enforcement have shaped the way the field is defined and practiced today. These key figures also happen to be predominantly White. To give grounds for this claim, this study drew from journal articles, textbooks, and book chapters specifically dealing with the history of forensic anthropology to identify the most often cited forbearers of the field. The most encountered names include Dwight, Hrdlička, Hooton, Stewart, Terry, Angel, Krogman, Todd, Kerley, and Snow, among other prominent figures, many of whom lend their names to special awards given out by the Anthropology Section or Kerley Foundation. This study contends that these individuals comprise the traditional pantheon of forensic anthropology's luminaries, yet do not represent the diversity of our field, past or present. To ameliorate this gap, this study highlights people of color in forensic anthropology's foundational history that are often overlooked in texts on the subject. This study adds early pioneers such as W. Montague Cobb, Charles Warren, Caroline Bond Day, Tadao Furue, Kazuro Hanihara, Mahmoud El-Najjar, and others to the existing, well-recognized, and widely taught pantheon of forensic anthropology's founders. Their biographies, legacies, and contributions to the field are followed by a critique of why such an endeavor was required in the first place and of the challenges in compiling a robust list of names to include. Moreover, this study on this project includes a diverse set of early career scholars of color, which is intended to symbolize the current generation lifting up those from prior generations who may have been historically excluded. Such a colorization of our history coincides well with this year's annual conference theme of responding to and surpassing the needs of a modern forensic world and is intended to celebrate 50 years of anthropology at the AAFS in a more diverse and inclusive way. This presentation complements another symposium during these meetings called "Pioneers of Color: A Diverse Narrative of Forensic Anthropology," which colleagues are encouraged to attend.

### History of Forensic Anthropology, Diversity and Inclusion, Decentering Whiteness

## A34 Contributions of Master's Degree Programs to Forensic Anthropology Education, Training, and Professionalization

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**Learning Overview:** After attending this presentation, attendees will better understand the roles of master's-level programs, and their associated forensic anthropology laboratories, in education, training, and professionalization of forensic anthropology graduate students.

**Impact Statement:** This presentation will impact the forensic science community by discussing: (1) the advantages and challenges of the increased availability of specialized forensic anthropology education and training at the master's level, and (2) how master's-level programs contribute to the training and professional development of students.

For most of the 20th century, the dominant model of education and training within forensic anthropology has been a broad, four-field undergraduate education, followed by specialization in biological anthropology or bioarchaeology at the graduate level, and subsequent mentorship and hands-on training in forensic techniques and methodological approaches.

Increased recognition and utilization of forensic anthropologists, alongside the development of specialized theory, methods, and techniques for forensic contexts, has driven the need for focused education and hands-on training. In response, beginning in the 1970s, an increasing number of post-baccalaureate and graduate degree programs have filled this niche. This presentation highlights the contributions of such programs in forensic anthropology.

Three long-standing programs—California State University, Chico (Chico State), The University of Indianapolis (UIndy), and Mercyhurst University—that began accepting graduate students in 1975, 1996, and 2004, respectively, are used to emphasize broader trends in education at the master's level. Collectively, these three programs have produced over 250 graduates with master's degrees in Anthropology, Human Biology, and Forensic & Biological Anthropology, respectively. Of these graduates, more than 112 have completed or are currently enrolled in PhD or advanced professional degree (e.g., JD, MD) programs. Eighteen have been certified as Diplomates of the American Board of Forensic Anthropology (D-ABFS) with others in the process of obtaining this certification. Graduates have found employment within the forensic community in medical examiner and coroner offices, crime laboratories, agencies such as the Federal Bureau of Investigation (FBI), the Defense POW/MIA Accounting Agency (DPAA), and the International Commission on Missing Persons (ICMP), and as university faculty, while others have found related careers in anatomy, medicine, archaeology, and law.

The success of these programs is grounded in their ability to provide practical, hands-on training through specialized, laboratory-based classroom courses, student-focused research, and participation in forensic cases alongside their faculty mentors. Importantly, each institution began accepting forensic casework prior to graduate program formation (Chico: 1972; UIndy and Mercyhurst: 1991), which laid the foundation for graduate student training. These programs also offer continuing education courses to law enforcement and medicolegal professionals, which in turn promotes the use of forensic anthropology and a significant expansion in casework.

With the field's increasing popularity and visibility, external pressures to expand class sizes have become increasingly prevalent. In response, existing programs have taken steps to combat the negative consequences of producing a large number of graduates in a small, competitive field by promoting small class sizes, increasing professionalization opportunities, and promoting applicable skills within fields, such as biology, forensic science, and bioarchaeology. With an emphasis placed on training students in a diverse range of scientific methodologies, these programs facilitate job placements in other areas of forensic science or related professions.

The goal of master's-level degree programs should continue to be the integration of hands-on teaching, research, and casework, which provides young professionals with real-world forensic experience and prepares them for entering the workforce or pursuing higher education. For some graduate students, master's-level training is the only opportunity they will have to develop their skills participating in forensic casework opportunities. Therefore, development of a masters-level certification will provide an additional avenue for developing professionals to demonstrate competency and commitment to professional involvement in the field.

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### Forensic Anthropology, Master's Programs, Professionalization

## A35 *Daubert*: Lip Service or a Substantive Change in Forensic Anthropology?

Kate Lesciotto, PhD\*, Sam Houston State University, Conroe, TX

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how the United States Supreme Court decision *Daubert v. Merrell Dow* has impacted forensic anthropological research.1

**Impact Statement:** This presentation will impact the forensic science community by presenting data on the subject matter, methods, and relevant legal standards discussed in forensic anthropology articles published in the *Journal of Forensic Sciences*.

This project examines trends in forensic anthropology articles, with specific emphasis on whether the 1993 United States Supreme Court *Daubert v. Merrell Dow* decision on the standard for admissibility of expert witness evidence has changed the landscape of forensic anthropology research. In the wake of *Daubert*, many forensic anthropologists anticipated significant impacts on the admissibility of forensic anthropological expert witness testimony, with numerous articles citing *Daubert* as motivation to create more objective and statistically backed methods.

This bibliometric study evaluated 1,142 peer-reviewed forensic anthropology articles published between 1972 and 2020 in the *Journal of Forensic Sciences (JFS)*. Data collected included the topic, article type, data type, inclusion of accuracy and error testing, and discussion of relevant legal standards, in order to address the following research questions: (1) re there general trends in the topics of published JFS anthropology articles?; (2) Has there been a change in how frequently publications discuss or cite legal standards, including *Daubert*?; (3) Did the field of forensic anthropology respond to *Daubert* by advancing toward more objective methods?; and (4) Has the field's overall discussion and awareness of *Daubert* initiated a shift in how forensic anthropology research is conducted?

Forensic anthropology articles published in JFS have risen from 1 article in 1972 to a high of 70 in 2015, with a decrease to 34 in 2020. The biological profile was the most common topic (36%), followed by taphonomy and/or postmortem interval (12%), and trauma (11%). Legal standards were discussed in 123 articles, with 85 specifically citing the *Daubert* decision. Articles as early as 1982 referenced relevant legal standards, with the first specific reference to *Daubert* occurring in 2005. Chi-squared trend tests showed a significant increase in the proportion of articles that referenced legal standards (including *Daubert*) over time ( $\chi^2=19.538$ ;  $p < 0.001$ ), indicating a growing awareness of the potential implications for forensic anthropology.

Using the biological profile articles as a case study, a chi-square trend test revealed no significant decrease over time in the proportion of studies using subjective data ( $\chi^2=2.509$ ;  $p=0.1132$ ), suggesting that *Daubert* has not significantly driven forensic anthropology research toward more objective methods. However, there was a significant increase in the proportion of studies that performed intra- and inter-observer error studies ( $\chi^2=12.264$ ;  $p < 0.001$ ).

Qualitative analyses were also performed to further evaluate the articles that did reference legal standards ( $n=123$ ). Thematic analysis revealed that articles commonly referenced legal standards while providing a general background of a legal framework (e.g., listing the *Daubert* guidelines) (50%) or as justification for conducting the study (19%). Forty-three of the articles (35%) specifically contextualized the results of the study within the relevant legal framework, such as explaining how the study met one or more of the *Daubert* guidelines.

While no articles disputed the influence of *Daubert* and other relevant legal standards on forensic anthropology research, the data demonstrate that authors are commonly using *Daubert* to point out deficiencies in previous research and justify a new study or publication. However, the field of forensic anthropology still needs to take further steps toward advancing more objective, quantifiable, and statistically backed methods and research to bring the field more into compliance with the *Daubert* guidelines.

### 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.

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### Forensic Anthropology, *Daubert*, Legal Standards

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## A36 50 Years of Taphonomic Research: From Death's Acre to a Global Footprint

*Sophia Mavroudas, MA\**, Texas State University, San Marcos, TX; *Dawnie Steadman, PhD*, University of Tennessee, Knoxville, TX; *Katie Zedjlik, PhD*, Western Carolina University, Cullowhee, NC; *Daniel Wescott, PhD*, Texas State University, San Marcos, TX; *Timothy Gocha, PhD*, Texas State University, San Marcos, TX; *Sheree Hughes, PhD*, Sam Houston State University, Huntsville, TX; *Gretchen Dabbs, PhD*, Southern Illinois University, Carbondale, IL; *Melissa Connor, PhD*, Colorado Mesa University, Grand Junction, CO; *Jodie Ward, PhD*, University of Technology, Sydney, New South Wales, Australia; *Erin Kimmerle, PhD*, University of South Florida, Tampa, FL; *Jane Harris, PhD*, Northern Michigan University, Marquette, MI; *Shari Forbes, PhD*, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada; *Anthony Falsetti, PhD*, George Mason University, Fairfax, VA

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**Learning Overview:** This presentation will inform attendees as to the history of outdoor decomposition facilities and the evolution of taphonomic research over the past 50 years.

**Impact Statement:** This presentation will impact the forensic science community through the presentation of history pertinent to the field of forensic science, particularly forensic taphonomic research.

The first outdoor human decomposition research facility was established by Dr. William Bass in 1981 at the University of Tennessee in Knoxville, TN. The Anthropology Research Facility, originally one acre in size, is located on a bluff top overlooking the Tennessee River. With the establishment of this facility, Dr. Bass initiated the important connection between time-since-death estimation and questions pertinent to the field of forensic anthropology. Many early research questions focused on single environmental variables (e.g., decomposition rates in sun or shade, clothed and unclothed, etc.). More recently, however, forensic anthropology has grown as a discipline such that taphonomic research is more holistic and multidisciplinary. This has led to research at outdoor decomposition facilities that investigates much more complex relationships between the decomposing body and its environment in diverse landscapes across the United States in North Carolina, Texas, Illinois, Colorado, Florida, Michigan, and Virginia.

To date, there are nine working facilities within the Consortium for Anthropological Research in Taphonomy (CART) within the United States in addition to one each in Australia, Canada, and the Netherlands. Most of these facilities are run by female anthropologists; however, several are now led by specialists in other disciplines, including geomatics, chemistry, and archaeology. The focus on hypothesis-driven scientific research and pedagogy has grown exponentially since 1981, and these facilities now play an important role in the field of forensic science providing real-world laboratory environments, research material, opportunities for student research at all levels, and documented modern skeletal collections.

This presentation will cover the history and current research of decomposition facilities across the world from the original facility in Tennessee through to the most recent facility in Quebec, Canada. This presentation will focus on the unique specialties of each facility, cross-disciplinary research that has grown out of these facilities, and where the consortium sees the future of forensic taphonomy research headed. Special emphasis will be placed on the evolution of taphonomic research design and scope across the five decades.

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### Taphonomy, Outdoor Decomposition Facilities, Forensic Science History



## A37 **Beatings, Broken Bones, Bullet Holes, and 3D Prints: The Evolution of Bone Trauma From the Morgue to the Virtual World/Space**

*Ericka N. L'Abbe, PhD\**, University of Pretoria, Pretoria, Fauteng, South Africa; *Alison F. Ridel, PhD*, University of Pretoria, Pretoria, Fauteng, South Africa; *Charlotte Theye, MSc*, University of Pretoria, Pretoria, Fauteng, South Africa; *Rudolph G. Venter, MMed*, Stellenbosch University, Stellenbosch, Western Province, South Africa; *Alieske Hagg, MSc*, University of Pretoria, Pretoria, Fauteng, South Africa; *Pearl Bothma, BSc*, University of Pretoria, Pretoria, Fauteng, South Africa; *Angelique Carmichael, BSc*, University of Pretoria, Pretoria, Fauteng, South Africa; *Clarisa van der Merwe, MSc*, University of Pretoria, Pretoria, Fauteng, South Africa; *Steven A. Symes, PhD*, Office of the State Medical Examiner of Mississippi, Pearl, MS

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the use of 3D surface scanning, micro-Computed X-Ray Tomography (micro-XCT) scanning, 3D mesh creation and 3D printing of bone trauma.

**Impact Statement:** This presentation will impact the forensic science community by presenting a history of bone trauma in the United States and how 3D technology can improve the application of bone trauma in laboratory, classroom, and courtroom.

Worldwide, a high burden of trauma exists in both clinical and medicolegal disciplines. While not all bone fractures cause death, a fracture can be related to the cause of death, which is important in any medicolegal investigation. The interpretation of traumatic injury to bone is a critical component of a forensic anthropological case report and in clinical applications.

The interpretation of bone trauma as part of a forensic anthropologists toolkit began in the 1990s out of the Office of the State Medical Examiner in Memphis, TN. During postmortem investigations, forensic anthropologists resected broken bones, cleaned, reconstructed, and used them, in association with soft tissue and prior knowledge, to assist a forensic pathologist in interpretations of cause and manner of death.<sup>1</sup> In the majority of cases, the resected bone was retained as evidence and used either in the courtroom or the postgraduate classroom. Bone trauma analysis expanded an anthropologist's contribution to forensic investigations, but education in bone trauma has faltered nationally and internationally, with varied ethics and legislation around the retainment of human bone. Forensic anthropology postgraduate students and practitioners struggle with practical experience in bone trauma analysis as few illustrative examples exist and, due to the nature of biological materials and ethics, access to these specimens are limited to certain facilities.

As part of the Bakeng se Afrika ("for Africa") project, a total of 67 bone trauma specimens were micro-XCT scanned from 2019 to 2020 at the Nuclear Energy Corporation South Africa (NECSA), and include both crania (n=48) and postcrania (n=17) of adults and two juveniles (n=1 cranium and n=1 rib cage). The specimens are comprised of anonymized and donated resected bone and/or complete skulls from closed cases in both the United States and South Africa. The distribution of trauma types includes blunt force trauma (n=26), ballistic/projectile trauma (n=25), sharp force trauma (n=4), healed injuries (n=9), and thermal alterations (n=3). Two femora have projectile trauma with known weapons, along with two cases of substantiated child abuse. All digital volumes of these specimens were processed into 3D meshes, providing a way in which to generate high-quality 3D prints of traumatic injury.

Digital imaging technology provides an opportunity to improve education in bone trauma analysis, as a 3D mesh or print of a bone is not a biological material and can be ethically used in both undergraduate/postgraduate education and workshops. While micro-XCT and professional expertise associated with them are not readily available in many laboratories, the use of 3D surface scanners can effectively capture the shape and size of the morphology of a traumatic injury in a non-invasive way, thereby easing documentation, data sharing, collaborative analysis, and 3D printing. 3D imaging and printing provide practitioners, students, and the public an opportunity to handle broken bones in both undergraduate and postgraduate classrooms, workshops, and the courtroom.

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

- <sup>1</sup> Dirkmaat, D.C.; Cabo, L.L. Forensic Anthropology: Embracing the New Paradigm. In: *A Companion to Forensic Anthropology*, eds. Dirkmaat, DC, Wiley-Blackwell: UK. 2012:3-41.

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### **Bone Trauma, 3D Prints, Forensic Science Education**

## A38 Situating Activism In Forensic Anthropology: Historical Understandings of Advocacy in the Discipline

Raphaela Meloro, MA\*, University of Florida, Department of Anthropology, Gainesville, FL; Isis Dwyer, MA, University of Florida, Department of Anthropology, Gainesville, FL; Thomas Delgado, BA, California State University-Chico, Department of Anthropology, Chico, CA

**Learning Overview:** After attending this presentation, attendees will understand how the role of activism in the field of forensic anthropology has developed and shifted over time.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into current and future understandings of the role of activism within forensic anthropology and science at large.

In the wake of an escalating civil rights movement with severe government responses, many disciplines have had to consider the way in which racism and discrimination against minority groups exists in their histories and understand the roles that activism and advocacy currently play in the field. This is of particular relevance for the field of forensic anthropology in the wake of public backlash against the use of unprovenanced human remains in skeletal collections used for teaching and research. Public perception of forensic anthropology would suggest a decline in activism and advocacy over time, as demonstrated by the responses of key leaders in the American Academy of Forensic Sciences (AAFS) and American Board of Forensic Anthropology (ABFA) to the Black Lives Matter movement and backlash regarding the storage of human remains from the 1985 MOVE bombing in public news.<sup>6,9</sup> Yet, forensic anthropologists are not, and have not been, shy about promoting justice via activism over the formative years of our practice. Indeed, the direct involvement of anthropologists in international humanitarian crises over the years serves as a stark reminder of our role. Human rights work by forensic anthropologists has continued on in the present with the field's engagement in modern international humanitarian crises and in the re-examination of historic cases, such as the 1921 Tulsa Race Massacre and the identification of Native American remains from historic boarding schools.<sup>1-5,11</sup> Nonetheless, there have been views expressed from within the field that are opposed to the role of anthropologist as advocate.

This presentation aims to situate this issue in the historical context of the discipline and examine the role of activism in forensic anthropology over the past 50 years. By examining contributions of notable forensic anthropologists to historic and modern rights movements, such as the investigations of human rights violations in Nazi Germany and South America, this presentation demonstrates the shifting nature of activism in the field over time.<sup>1,2,4,5,7,9</sup> Results of this retrospective review suggest that the definition of activism has changed in the field over time, and this presentation captures this change by differentiating between humanitarian efforts, activism, and reactionary efforts to current events. A paradigm shift initiated by diversification and growth in the field requires a reexamination of the role of activism in forensic anthropology.<sup>8-11</sup>

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9. Tallman, S. (2020). Opinion: The Forensic Sciences Have a Diversity, Inclusion Problem. *Forensic*. <https://www.forensicmag.com/569912-Opinion-The-Forensic-Sciences-Have-a-Diversity-Inclusion-Problem/>.
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11. Waxman, O.B. (2021). 100 Years After the Tulsa Race Massacre, Meet the Forensic Anthropologist Searching for Victims' Remains. *Time*. <https://time.com/6050742/tulsa-massacre-victims-dig/>.

### Activism, Humanitarian Action, Civil Rights

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## A39 Standards Development Activities in Forensic Anthropology

*Gregory E. Berg, PhD\*, Defense POW/MIA Accounting Agency, Honolulu, HI*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of forensic anthropology.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to forensic anthropology. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

Even during this trying time of a global pandemic, the forensic anthropology community continues to strive forward and make progress in the Standards world. Specifically, the Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them, often as an American National Standard or an American Society for Testing and Materials Standard, among others. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards. This presentation will cover the latest advances made by the OSAC and the other SDOs in regard to forensic anthropology.

During this presentation, updates related to standards development in forensic anthropology will be presented. These include: (1) recent standards that have been added to the OSAC Registry (none yet, but three are under consideration); (2) OSAC-proposed standards (nine documents in process at OSAC and the Academy Standards Board [ASB]); (3) published standards from the ASB that have yet to go through the Registry approval process (as noted earlier, these are under consideration for OSAC Registry inclusion and deal with the topics of Stature Estimation, Facial Approximation, and Sex Estimation); (4) priorities for new documents or work products (six documents entering the queue for development on items such as Qualifications, Documentation, Proficiency Testing, Isotope Analysis, etc.), as well as other highlights. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### Forensic Anthropology, Standards, OSAC

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## A40 Fatal Environments: Survey Results and Best Practices for Human Donor Programs and Body Farms Around the World

*Kelsee Hentschel-Fey, MA\*, University of South Florida, Tampa, FL; Gennifer Goad, MA, University of South Florida, Tampa, FL; Erin Kimmerle, PhD, University of South Florida, Tampa, FL*

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**Learning Overview:** After attending this presentation, attendees will better understand the ways in which outdoor decomposition facilities are managed around the world, the diversity of research and innovation taking place in these facilities, and the changing practices within living human donor programs.

**Impact Statement:** This presentation will impact the forensic science community by bringing attention to the multidisciplinary nature of outdoor decomposition facilities, the problems associated with the application of experimental research, and the unique familial and kinship research opportunities afforded by skeletal collections.

Human donation programs and outdoor research facilities (“body farms”) enable critical research necessary for method testing and development in areas of identifying unknown persons, locating burials, and trauma interpretation, all of which can be critical to solving cases and prosecuting offenders. These human donations are kept in perpetuity and accessioned into donated skeletal collections, which provide a wealth of opportunity for students and scholars in need of ethically sourced research collections with known demographic and life history information.

To date, there are 12 programs utilizing a human donation program for forensic research at an outdoor decomposition facility. Each of the 12 directors participated in a jointly written survey to share information about their donation policies, available research collections, and current trends in research. Survey results from the 12 facilities include 9 that are domestic and 3 international: (1) Forensic Anthropology Research Facility (FARF), Texas State University; (2) Southeast Texas Applied Forensic Science (STAFS) Facility, Sam Houston State University; (3) Complex for Forensic Anthropology Research (CFAR), Southern Illinois University; (4) Facility for Outdoor Research and Training (FORT), University of South Florida; (5) Forensic Research Outdoor Station (FROST), Northern Michigan University; (6) Forensic Osteology Research Station (FOREST), Western Carolina University; (7) Forensic Investigation Research Station (FIRS), Colorado Mesa University; (8) Anthropology Research Facility (ARF), University of Tennessee; (9) Forensic Science Research and Training Laboratory, George Mason University; (10) Australian Facility for Taphonomic Experimental Research (AFTER), University of Technology, Australia; (11) Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology (ARISTA), Amsterdam University Medical Centers, The Netherlands; and (12) Research on Experimental and Social Thanatology (REST[ES]), University of Quebec, Canada.

Results of this survey found that research conducted at these facilities is multidisciplinary, combining anthropology, archaeology, legal medicine, geology, chemistry, biochemistry, geochemistry, remote sensing, botany, genetics, engineering, and art. A wide range of studies have been and are continuing to take place, including remote sensing techniques for locating burials, evaluation of chemical changes in soils, DNA degradation, mineral variation in tumor growth, facial recognition, and generalized postmortem decomposition research. This survey also found interesting and unique trends common to multiple facilities that differ from forensic casework, illustrating some of the inherent challenges in applying experimental research to real-world scenarios.

The Facility for Outdoor Research and Training at the University of South Florida (USF-FORT) will be used as an in-depth case study to illustrate some of the trends found in this survey. Created in 2014, the program has over 100 donors and 250 registered pre-donors to date. Reasons for donation vary and frequently include individual perceptions of environmental sustainability, support for the forensic community or education, and/or low-cost/affordability. Further, a surprising trend in donation practices includes family registrations: at least 30 families have registered with the program, including spousal/life partnerships, parents/children, grandparents/grandchildren, siblings, and second-degree relatives. This suggests forensic facilities are used by families as alternatives to traditional final disposition sites like cemeteries or mausoleums. As forensic facilities continue to be established internationally, these perceptions are relevant to facility directors and donation coordinators who are involved with end-of-life care and planning. Further, permanent donation programs comprised of households and multi-generational family lineages may offer unique research avenues regarding kinship, including heritability of skeletal traits, isotopic analyses, and genetic analyses.

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### Forensic Anthropology, Decomposition, Collaboration

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## A41 F-Track: A Model for Forensic Professionalization

Joanne Devlin, PhD\*, University of Tennessee, Knoxville, TN; Giovanna Vidoli, PhD, University of Tennessee, Knoxville, TN; Lee Jantz, PhD, University of Tennessee, Knoxville, TN; Mary Davis, MSc, University of Tennessee, Knoxville, TN; Dawnie Steadman, PhD, University of Tennessee, Knoxville, TN

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**Learning Overview:** After attending this presentation, attendees will have a model program and a successful blueprint for professional development in forensic anthropology graduate programs. This roadmap follows educational accreditation standards without the potential accreditation roadblocks.

**Impact Statement:** Recent discussions have focused on accreditation of forensic anthropology programs in higher education.<sup>1</sup> The Department of Anthropology at The University of Tennessee, Knoxville has an established undergraduate concentration in forensics for anthropology majors; however, no such program formally exists at the graduate level. Unfortunately, an accredited forensic program via the Forensic Science Education Programs Accreditation Commission (FEPAC) or other accreditation body is improbable given other university and department priorities. However, it is still a departmental responsibility to establish programmatic standards and to provide graduate students pathways to professional success. This approach must extend beyond basic classroom instruction, opportunistic experiences, and thesis or dissertation-related research. Further, in light of the upcoming American Board of Forensic Anthropology (ABFA) forensic anthropology certification available to Masters-level students, systems that provide students the opportunities to learn, and practice, how to be solid forensic practitioners and professionals are critical. The absence of a waiting period before seeking Diplomate-ABFA (D-ABFA) certification after earning a degree further underscores the importance of incorporating a system like the one outlined in this presentation into established anthropology graduate programs.

Well-rounded educational programs aim to blend classroom-based sessions where methods, techniques, and best practices are conveyed, with practical professional development through participation in applied opportunities, training courses, apprenticeships, and internships. However, this format—classroom with practical opportunities—may not be sufficient to develop knowledgeable and well-rounded forensic professionals. Graduate students deserve a structured program that provides a pathway to blend academic requirements with professional and practical milestones. As a result, in the fall of 2017, the Forensic Anthropology Center (FAC) at The University of Tennessee implemented Forensic track (F-track), a voluntary program designed to fulfill suggested competencies. Two years later at the American Academy of Forensic Sciences (AAFS) annual meeting, Langley and Tersigni-Tarrant shared seven core competencies and associated Entrustable Professional Activities for forensic anthropologists to sustain lifelong learning, professional growth, and personal growth.<sup>1</sup> University of Tennessee Anthropology's F-track program provides a model to accomplish these competencies.

F-track currently has two major branches. One is aimed at providing the pathway for access to, and success in, forensic case work, starting with competency tests, required coursework, and then transitioning to primary analyst level. The other branch is geared toward professionalization by nurturing confidence and capabilities in science communication, presentations, and inter-professional instruction. This branch is equally important and provides opportunities to work on communication skills, professionalism, and inter-professional collaborations. Each branch is comprised of several modules. Successful completion of a module indicates a participant has acquired and demonstrated knowledge of practices relevant to case work and professional development. Several modules require understanding of Standard Operating Procedures (SOPs) used at the FAC. F-track provides the milestones necessary for motivated students to secure forensic training and experience while developing and nurturing their professional capabilities. Importantly, each participating student will have a documented and detailed training log that they can include in their application for certification upon graduation. The F-track model can be adopted by all who seek a formal, structured system of professional graduate training without external programmatic accreditation that may be unobtainable for most anthropology departments.

### Reference(s):

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### Graduate Student Training, Professionalization, Accreditation

## A42 The Readability of Forensic Anthropological Abstracts

Donovan Adams, PhD, University of Central Florida, Orlando, FL; Clairia Ralston, MS\*, University of Nevada, Las Vegas, NV; Kelly McGehee, BA, University of Central Florida, Orlando, FL

**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the factors contributing to the readability of forensic anthropological abstracts and how these factors impact the ability of both anthropologists and the general public in understanding academic research.

**Impact Statement:** This presentation will impact the forensic science community by providing information regarding the complexity of abstracts and providing potential avenues for science communication improvement.

The intent of abstracts is to provide a concise, yet thorough, summary of an article. Oftentimes these are the only sources of information, whether through lack of subscription access or through conference proceedings. Additionally, these serve as the entry to a publication as both researchers and non-researchers search for relevant information. Effective science involves effective communication; otherwise accurate interpretation, replication, and knowledge-building is hampered. The present research examines the readability, defined here as the processing and understanding of a text, of anthropological abstracts.<sup>1</sup>

Abstracts from 2010-2021 were gathered from PubMed® using the pubmedR2 package and the American Academy of Forensic Sciences website. These included Anthropology papers in the Journal of Forensic Sciences (JFS; n=574), Forensic Anthropology (FA; n=85), and the AAFS Conference Proceedings (AAFS; n=1,578). As a comparative sample to broader biological anthropology, abstracts were collected from the American Journal of Physical Anthropology (AJPA; n=2,034). The program Tool for the Automatic Analysis of Lexical Sophistication (TAALES) and packages koRpus and quanteda.textstats were used to calculate various readability and lexical indices.<sup>3-6</sup> The following questions were addressed: (1) are abstracts becoming more complex over time?; (2) are forensic anthropological abstracts more complex compared to the average bioanthropological abstract?; (3) do certain aspects of writing contribute to more complex abstracts?; and (4) how do the answers to these questions inform better science communication?

Preliminary results suggest abstracts in AAFS are the most complex and JFS are the least complex in readability measures. Of the journal abstracts, FA appears to be more difficult in readability. AAFS appears to be decreasing in readability while AJPA remains the most relatively consistent. Abstracts in JFS exhibit variability in text familiarity, imageability, and concreteness across years, though this may be related to smaller sample size. On average, however, JFS had the highest and FA had the lowest text familiarity, imageability, and concreteness, respectively (JFS had terms that were more familiar and less abstract). Comparisons between journals for these measures were significant, but effect size was small. Measures of word range and frequency and bigram proportions in academic, news, fiction, and magazine corpora indicate most abstracts are written more similar to other academic texts compared to non-academic sources. Although AAFS shows a higher similarity to non-academic corpora, this may, in part, be due to the longer length of AAFS abstracts, which allows for greater possibility of similarity. These results agree with traditional measures like Flesch's Reading Ease and Flesch-Kincaid Readability Score that abstracts are generally written for at least a college graduate level.

These preliminary results suggest anthropological abstracts, especially those of conference proceedings, are inaccessible, particularly to those with minimal to no training in (forensic) anthropology. Notably, AAFS consistently had values in each of the indices that are reflective of lower readability. While practitioners may argue these results fit their audience, we must consider that we should write abstracts (any scientific writing) for the broader public. Research is spread across the internet, in sometimes nefarious ways, so it is important that anthropologists ensure that, at minimum, abstracts are written for everyone. This presentation will discuss avenues for improved science communication regarding jargon, writing, text length, and sentence construction.

### Reference(s):

1. Crossley, S.A.; Skalicky, S.; Dascalu, M. 2019. Moving beyond classic readability formulas: New methods and new models. *Journal of Research in Reading*. 42(3-4):541-561.
2. Aria, M. 2020. Package "pubmedR." Accessed from <https://cran.rproject.org/web/packages/pubmedR/pubmedR.pdf>.
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### Abstract Readability, Science Communication, Forensic Anthropology

## A43 Estimating the Postmortem Interval (PMI) in High Desert Climates

Mackenzie Sullivan, BA\*, University of Nevada-Reno, Reno, NV; Marin Pilloud, PhD, University of Nevada-Reno, Reno, NV; Mary Megyesi, PhD, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI; Laura Knight MD, Washoe County Regional Medical Examiner's Office, Reno, NV

**Learning Overview:** After attending this presentation, attendees will better understand how regionally specific methods to estimate PMI can be created and how well they perform.

**Impact Statement:** This study will impact the forensic community by comparing different methods of scoring decomposition to estimate PMI.

Estimating PMI is challenging due to the variety of factors that affect decomposition rates (e.g., temperature, sun exposure, humidity, animal scavenging). One commonly used method is that of Megyesi et al.<sup>1</sup> This method utilizes a decomposition scoring system of visual indicators, which when totaled, provide the Total Body Score (TBS). This score is then correlated with Accumulated Degree Days (ADD) to estimate the PMI. An alternative method of scoring TBS by Connor et al. incorporates desiccation rates as regionally appropriate for western Colorado.<sup>2</sup> Connor and colleagues conclude that incorporating the Total Body Desiccation Score (TBDS) may help refine PMI estimates in areas where remains desiccate. This project recreates the research of Megyesi et al. and Connor et al. on a sample of cases from northern Nevada to identify if a regionally specific equation and/or one that incorporates desiccation rates can better predict PMI.

Cases of identified decedents were obtained from the Washoe County Regional Medical Examiner's Office (WCRMEO) in Reno, NV. The PMI was determined by the difference of days between when the decedent was last seen and the day the decedent was recovered. Only cases including full-color, high-quality photographs of each region of the body were accepted, and all cases had macroscopic signs of decay at the scene and/or in the autopsy. The TBS and TBDS were scored for each set of remains ( $n=99$ ) with observer error checked. Of the total sample, the majority were found indoors ( $n=93$ ) and between March 1 and August 31 ( $n=67$ ). The ADD was obtained by averaging the high and low daily temperature collected from the nearest weather station to the decedent's location and totaling each daily average for all days in the PMI. The PMI in this sample ranged from 1 to 75 days and the ADD ranged from 0 (all daily temperature averages below 32° Fahrenheit) and 3,741. The TBS ranged from 3 to 23; the TBDS ranged from 0 to 40.

A linear regression model was created specific to northern Nevada to estimate PMI based on the two different decomposition scores as well as the ADD for each case. The models were created using a training set ( $n=80$ ); a testing set was used to test the models ( $n=19$ ). For the test set, this study tested its Adapted Megyesi score (based on TBS and ADD specific to the cases of the WCRMEO), a model based on the TBDS, and the original formula created by Megyesi et al. to estimate PMI. All analyses were conducted in the R statistical computing environment.<sup>3</sup>

Between the two new models, the Adapted Megyesi Model provides more accurate predictions of PMI (correct in 57.9% of test cases), whereas the model based on TBDS was accurate in 47.4% of cases. The Original Megyesi model was most accurate at 84%. However, the Original Megyesi model also had the largest standard error, over six times that of the new models created here, which allowed it to be more accurate but much less precise.

Based on this research, the Original Megyesi Model held the most predictive value for estimating PMI. However, the two models built using a regionally specific sample offered a narrower error range and were more precise in PMI estimates. Therefore, an accurate and more narrowly precise PMI estimation for local medicolegal death investigations (i.e., a regionally specific equation) could be constructed with a larger sample size and further investigation into mitigating variables. This research and continued efforts to study PMI will aid in creating more refined and regionally appropriate methods.

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2. Connor, M.; Baigent, C.; Hansen, E.S. Measuring Desiccation Using Qualitative Changes: A Step Toward Determining Regional Decomposition Sequences. *J Forensic Sci.* 2019;64(4):1004-11.
3. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL <http://www.R-project.org>, 2019.

### Decomposition, Desiccation, Taphonomy

## A44 Toward a Better Understanding of the Effects of Leaching and Diagenesis on $^{87}\text{Sr}/^{86}\text{Sr}$ of Human Hair Keratin

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**Learning Overview:** Attendees will learn how diagenesis/postmortem burial contexts and leaching protocols can alter the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios recovered from human hair keratin.

**Impact Statement:** This presentation will inform the forensic science community how hair keratin cleaning methods commonly employed by forensic scientists influence the Sr isotope composition. This presentation will give guidance as to which cleaning methods are preferred.

The application of isotope analysis to aid human identification processes in forensic cases is becoming more common. In contrast to dental enamel, hair and nail keratin can potentially provide valuable insights into recent diet and health status (e.g., C-N-S), and geolocation (e.g., Sr-Pb). Recent studies, however, have shown that it is difficult to separate diet-related endogenous Sr-Pb signatures from environment-specific exogenous signatures in human hair keratin. Inevitably, the effectiveness of hair keratin as a means of evidence for reconstructing recent mobility patterns depends greatly on its susceptibility to diagenetic alterations (e.g., burial context).

To gain a better understanding of the optimal pre-treatment of hair keratin samples and the applicability of hair keratin samples in various forensic contexts, an experimental leaching project was combined with actualistic experiments at the Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology (ARISTA) facility in the Netherlands and human hair samples recovered from Undocumented Border Crossers (UBCs) provided by the Pima County Office of the Medical Examiner in Arizona. The samples from ARISTA consist of pre-burial and post- clandestine-burial samples, which were then compared to investigate if the exogenous signature can be successfully removed, and the endogenous (pre-burial) signature recovered. The UBC samples consist of hair samples recovered from individuals found above ground. Here, the leachates of every cleaning step and a subsample of hair were collected to examine during which cleaning steps (exogenous) Sr is released.

The data show a close connection between hair color (i.e., the amount of melanine present in the sample) and the effectiveness of the leaching protocol on retrieving the antemortem Sr isotope composition of hair keratin. More importantly, following previously reported data from FARF (Forensic Anthropology Research Facility) in Texas, the data from the actualistic experiments at ARISTA and the data from the UBCs accentuate the extreme difficulty or even impossibility of recovering the hair's biogenic Sr isotope compositions after 306 days of burial, despite the application of the most effective pre-treatment protocol (2:1 MC-MQ-0.1M HCL-MQ).<sup>1</sup> The UBC leaching experiment demonstrates the differences in effectiveness of cleaning steps and during which steps Sr was released from the human hair.

### Reference(s):

- <sup>1</sup>. Kootker, L.M.; von Holstein, I.C.C.; Broeders, J.; Wescott, D.J.; Davies, G.R.; Mickleburgh, H.L., 2020. The effects of decomposition and environment on antemortem H-Pb-Sr isotope compositions and degradation of human scalp hair: Actualistic taphonomic observations, *Forensic Science International* 312, 110336.

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### Strontium, Diagenesis, Human Hair



## A45 Diagenesis of Human Bone, Dental Elements, and Scalp Hair in Taphonomic Experiments: Implications for Isotope Analysis for Human Identification

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**Learning Overview:** After attending this presentation, attendees will have learned how taphonomic processes, diagenesis, and cleaning protocols can influence the strontium and lead isotope ratios recovered from human bone, dental elements, and scalp hair and the subsequent implications for forensic investigations using isotope analysis. Further, this presentation will provide recommendations on which human tissues to target for forensic isotope analyses for provenancing purposes.

**Impact Statement:** This research aims to contribute to the forensic science community by examining how taphonomic processes and diagenesis affect the recoverability of pre-burial isotope ratios in different human tissues. This presentation highlights the need for research into the effects of these processes on human samples frequently consulted in forensic investigations. This presentation will also caution the scientific community to further examine the effects of cleaning procedures on isotope signatures recovered from human tissues.

This presentation presents the results of a small-scale study on the effects of taphonomic processes and diagenesis on the strontium and lead isotope signatures of human scalp hair, human bone (iliac and tibia), and tooth enamel and dentin. The pre-placement and post-recovery Strontium-Lead (Sr-Pb) isotope data of five whole body donations studied at the Forensic Anthropology Research Facility (FARF) were compared. Two donations were buried in shallow graves, two were placed in shallow open pits, and one was first allowed to naturally mummify through solar and aeolian desiccation and was then buried. In addition to pre- and post-burial human tissue samples, soil samples of each grave and one control sample from an unused part of FARF were collected.

The Sr-Pb analysis of the hair samples from four of the donors demonstrated that it is difficult to recover pre-placement isotopic Sr signatures from human hair.<sup>1</sup> The results showed significant shifts in Sr-Pb isotope compositions between pre- and post-placement samples. The hair keratin Pb isotope composition shifted toward the Pb signature of FARF soil samples. The Sr isotope compositions of the hair keratin samples, however, were affected by the burial environment, but possibly also by the applied cleaning method. The experiment showed that hair keratin is highly susceptible to diagenetic alteration, but also highlights the possible effects of pre-treatment on the isotopic authenticity of hair keratin.

The iliac samples (i.e., spongy or trabecular bone) show inconsistencies in the recoverability of the pre-burial Sr-Pb isotope signatures. While the post-burial signatures of the buried donations are only slightly elevated over the pre-burial signatures, the post-placement signatures of open pit donations are significantly elevated. This could potentially be related to the exposure of these body donations to periodic heavy precipitation and temporary submerging during the experiment. Contrarily, burial in the clay-rich soil at FARF appears to have prevented this from happening in donations buried in shallow graves.

In contrast, the tibia and tooth enamel and dentin samples showed the most consistency out of all human samples used in this study, demonstrating less susceptibility to diagenesis compared to the hair and iliac samples. These more densely mineralized elements show good recoverability of the pre-placement isotope signatures in both burial and open pit contexts, and are thus deemed useful for usage in forensic investigations at least until three years after burial in similar environmental and climatological conditions.

In conclusion, this presentation highlights the need for continuous research into the effects of taphonomic processes, diagenesis, and cleaning protocols on Sr-Pb isotope compositions in human samples frequently consulted in forensic investigations. Based on this and previous studies, it is not recommended that human hair and/or spongy bone samples be used for Sr-Pb isotope analysis for provenancing purposes in cases involving buried individuals.

### Reference(s):

- <sup>1</sup> Kootker, L.M.; von Holstein, I.C.C.; Broeders, J.; Wescott, D.J.; Davies, G.R.; Mickleburgh, H.L. 2020. The effects of decomposition and environment on antemortem H-Pb-Sr isotope compositions and degradation of human scalp hair: Actualistic taphonomic observations. *Forensic Science International* 312, 110336.

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### Isotope Analysis, Diagenesis, Human Tissue Samples

## A46 The Reliability of Bone Features Commonly Used to Predict Saw Class Characteristics

*Alexandra Klaes, PhD\**, Washburn University, Topeka, KS; *Ashley Maxwell, PhD*, Washburn University, Topeka, KS; *Heather Garvin, PhD*, Washburn University, Topeka, KS; *Erin Chapman, PhD*, Erie County Medical Examiner, Buffalo, NY; *Lauren Butaric, PhD*, Des Moines University, Des Moines, IA

**Learning Overview:** After attending this presentation, attendees will understand the reliability of bone features that are used to predict inflicting tool type and saw class characteristics in sharp force trauma cases.

**Impact Statement:** This presentation will impact the forensic science community by testing whether or not commonly scored saw cut features found in bone can be reliably scored.

Determining saw class characteristics is dependent upon the macro- and microscopic analysis of features in cut bone. The entire suite of features present in a case is then used to predict class characteristics of the saw blade and teeth, such as power source, tooth set/size/shape, blade progress and direction, and/or inflicting tool type. While several studies have described these features, albeit often inconsistently, very few have tested the reliability of scoring these traits in cut bone.

To examine reliability, nine manual ( $n=4$ ) and mechanically ( $n=5$ ) powered reciprocating saws with variable class characteristics were used to create experimental complete ( $n=27$ ) and incomplete ( $n=18$ ) cuts in the long bones of a fleshed cadaveric sample ( $n=5$  individuals), thereby mimicking actual saw trauma cases. Following cutting and processing, each cut surface was given a unique randomized identification number to facilitate blind analysis. Two observers, with varying experience levels and not involved in the saw selection, cutting, or labeling/identifying, blindly scored each cut using a digital microscope with 3D capabilities. Each observer scored 13 features: breakaway notch, breakaway spur, exit chipping, entrance shaving, kerf flare, kerf shape (letter/number in incomplete cut sample only), false starts/incomplete cuts (scored incomplete cut sample only), harmonics, striation uniformity, polishing, pullout striations, tooth hop, and tooth imprint. Percent agreement was used to compare agreement between the observers.

Overall, reliability varied considerably by feature. Agreement was high ( $>85\%$ ) for presence of false starts/incomplete cuts (92.3%), breakaway spurs (88.9%), and exit chipping (85.3%). Scoring agreement was lower ( $<85\%$ ) for kerf flare (82.6%), pullout striations (72.7%), polishing (71.1%), breakaway notches (70.4%), harmonics (70.3%), tooth imprints (68.8%), tooth hop (66.7%), entrance shaving (65.9%), striation uniformity (60.5%), and kerf shape (46.7%). When disagreement occurred for entrance shaving, the more experienced observer scored more as present, while the less experienced observer scored more as absent. The opposite was true for disagreements on presence/absence of polishing. Nearly all of the disagreement on harmonics presence/absence occurred in incomplete cuts (90.9%). For tooth hop and tooth imprints, higher agreement was obtained in the incomplete cuts than the complete cuts primarily from agreeing upon absence of the features. Over half of the disagreements on kerf shape occurred between shape A versus shape B.

The results of this study suggest that some features used in the analysis of saw trauma can be reliably scored and, therefore, can be used to predict class characteristics of the inflicting tool; however, the vast majority of the features ( $n=10$ ) examined here had low levels ( $<85\%$ ) of observer agreement. Scoring inconsistencies means these features should be used with caution at present when applied to differentiating power source, tooth set, tooth shape, and/or tool type of the inflicting weapon. More research with a larger sample size is needed to confirm their reliability and utility in analyzing saw trauma to bone.

### Trauma Analysis, Sharp Force Trauma, Saw

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## A47 A Retrospective Analysis of Scavenging in Southern Nevada Forensic Anthropology Cases (2000–2021)

*Katharine Woollen, MS\*, University of Nevada-Las Vegas, Las Vegas, NV; Jennifer Byrnes, PhD, University of Nevada-Las Vegas, Las Vegas, NV*

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**Learning Overview:** After attending this presentation, attendees will better understand vertebrate scavenging patterns of human remains from southern Nevada.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of vertebrate scavenging, including prevalence, scavenger-type, and commonly impacted skeletal elements of human remains recovered across southern Nevada.

The Mojave Desert spans southeastern California and southern Nevada, with smaller portions within Utah and Arizona. This desert is considered the smallest and driest in the United States, exposing human remains to unique taphonomic processes in this harsh environment. One such taphonomic process is vertebrate scavenging. To date, no other forensic analysis has been conducted to explore the taphonomic patterns of vertebrate scavenging in southern Nevada. Coyotes, kit foxes, gray foxes, domestic dogs, bobcats, mountain lions, and domestic cats are among the most common carnivore scavengers in southern Nevada. There are several species of rodents as potential scavengers in this region as well, including the deer mouse, mice, rats, squirrels, and chipmunks. Carrion avian species in southern Nevada include various species of owls, falcons, and hawks, as well as turkey vultures, golden eagles, and bald eagles.

This presentation will offer an analysis of forensic anthropology case files from 2000–2021, which were reviewed for documentation of scavenging from the Clark County Office of the Coroner and Medical Examiner's Office (CCOCME) database. Of the 107 forensic anthropology cases reviewed, 30 cases (28%) had documented evidence of vertebrate scavenging. This included tooth pits, punctures, scores, and furrows attributed to carnivore dentition, as well as shallow scoring from vultures, and furrows created by rodents. The most commonly scavenged skeletal elements included the lower extremities (e.g., femora, fibulae, and tibiae), radii, ribs, and skull. These body regions, such as the thorax and long bones, are easy to grasp and provide access to high fat content, which is consistent with carnivore scavenging patterns. The least common body region with skeletal traces of scavenging were the hands, feet, sternum, and vertebrae. However, these least-scavenged skeletal elements are also the body regions most often missing from recovered cases. It is unknown if these body regions were consumed and/or carried off through scavenging activity, or if the skeletal elements were not recovered due to other taphonomic processes (e.g., weathering) since they are primarily small and/or high trabeculae bones. Comparative research conducted in the Sonoran Desert found that the small bones of the hands and feet are the least likely skeletal elements to preserve. Vulture scavenging data at the Forensic Anthropology Center at Texas State (FACTS) resulted in the skull and mandible being the first skeletal elements to be disarticulated. While these comparative data offer valuable insight into possible vertebrate scavenging patterns for southern Nevada, it is apparent that scavenging patterns in similar geographic areas, as well as at controlled facilities, are highly variable.

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### Scavenging, Southern Nevada, Forensic Taphonomy

## A48 Testing Blade Set Width Prediction From Minimum Kerf Width Using Fleshed Human Samples

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the accuracy and challenges in predicting saw blade set width from minimum kerf widths.

**Impact Statement:** This presentation will impact the forensic science community by presenting error rates for published blade width prediction equations.

In cases of dismemberment, saws often leave characteristic markings in bone that can provide evidence about the utilized tool, thereby facilitating investigations and impacting legal proceedings. One such characteristic, minimum kerf width, has been suggested to be a direct indicator of saw blade width. However, variables such as tissue presence, sawing methodology, and saw characteristics potentially impact minimum kerf width and consequently predicted blade width. This study aimed to test four previously published equations/rules for estimating saw blade width from minimum kerf width using a human cadaveric sample with intact soft tissue and a free sawing methodology that likely mimics actual forensic scenarios. Cadaveric samples were obtained through the Des Moines University Body Donor Program and the research was conducted with university approval following established Body Donor Program protocols.

Three hand saws and five mechanically powered reciprocating saws were used to create 48 measurable incomplete cuts in 20 limb segments (i.e., arm, forearm, thigh, leg) from five donors, taking care to vary the saws by limb segments and position along the diaphyses. Minimum kerf width was measured and collected using sliding calipers for each incomplete cut and compared to two equations presented by Norman et al. to predict blade set width, as well as blade width general rules presented by Symes and Cunningham and Holtrop.<sup>1-3</sup>

Supporting the Symes Rule, all blade set widths were within 0.02in (0.508mm) of the minimum kerf width. All minimum kerf widths were also less than 1.5x the blade set width, as suggested by Cunningham and Holtrop. Both of the Norman equations underestimated blade set width across the sample with an average absolute difference between the predicted and true blade set width of 0.40mm (both equations). Given the systematic underestimation, the predicted values were then compared to blade spine width measurements, which resulted in more balanced and accurate predictions (0.14mm average absolute difference). Through personal communications, however, Norman confirmed that blade width was measured at the cutting edge, and thus the higher accuracy with blade spine width is just a byproduct of spine width being less than blade set width. Despite small absolute differences, the percent error rates are high (27% average for blade set and 16% for blade spine) due to the relatively small size of kerfs. There was no pattern in accuracy for either equation across saw power type or teeth-per-inch. This study highlights the possible effects of soft-tissue presence and restraint conditions on minimum kerf width and the need for further testing in these conditions. Additionally, with such small measurements, even minimal rounding errors or measurement variability related to specimen modality, measurement tool, or measurement location can produce significant impacts on estimations—the effects of which need to be better understood when interpreting tool mark characteristics/measurements.

### Reference(s):

1. Norman, D.; Baier, W.; Watson, D.; Burnett, B.; Painter, M.; Williams, M. Micro-CT for saw mark analysis on human bone. *Forensic Science International*. 2018; 293: 91-100.
2. Symes, S. *Morphology of saw marks in human bone: Identification of class characteristics*. Doctoral Dissertation, University of Tennessee, 1992.
3. Cunningham, B.; Holtrop, W. *Woodshop Tool Maintenance Revised*. Chas. A. Bennett CO., Inc., Peoria, IL, 1974.

### Dismemberment, Sharp Force Trauma, Saw

## A49 The Evaluation of the Transition Analysis 3 (TA3) (Beta Ver. 0.8.0) Age-Estimation Program Using Two International Samples

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**Learning Overview:** After attending this presentation, attendees will understand the current capabilities and limitations of the TA3 program.<sup>1</sup>

**Impact Statement:** This presentation impacts the forensic science community by presenting the first systematic validation study of TA3 and contributing to ongoing method development using the TA3 traits.

In 2014, National Institute of Justice (NIJ) grant #2014-DN-BX-K007 was awarded for the development of an age-at-death estimation method using a suite of traits combined in a new analytical framework. In 2018, a reference dataset containing TA3 trait data for more than 1,700 documented individuals from four continents was completed. Although method and software development are ongoing, a version of the TA3 program has been released for public testing.<sup>1</sup>

The current program uses machine learning—a different approach for estimating age than the TA2 (ADBOU) software. For each case, a Random Generalized Linear Model (RGLM) predictor is trained using a portion of individuals in the TA3 reference sample and applied to generate an age estimate. The performance of the model in the training sample is visualized in a scatter plot and the sample size, correlation coefficient, and standard error are provided, along with the predicted point age estimate and 95% interval for the case analyzed.

In this study, 511 individuals from the University of Athens Human Skeletal Reference Collection ( $n=202$ ) and Santiago Subadult Osteology Collection (SSOC) ( $n=309$ ) were evaluated using the TA3 Trait Manual.<sup>2</sup> For estimating age, the TA3 program (beta Ver. 0.8.0) software interface and the source code were tested and explored by: (1) directly entering data into the software; and (2) executing the ta3.R analysis file modified into a loop to allow for batch analysis and extraction of associated analytical data.

Overall sample accuracy (documented age within the 95% interval) was 84.5%; however, critical differences exist for individuals under 40 years (57.0%, 73/128) versus those 40 years and older (93.7%, 359/383). Substantial age-estimation bias was present in the predicted point estimates with essentially all individuals (125/128) under 40 years overestimated by an average of 12.2 years. Between 40 and 80 years of age, a mix of over- and under-estimation occurred, with a slight trend of increased underestimation with advancing age. Precision (95% interval width) for individuals under the age of 40 was poor (average 27.4 years), while precision for individuals 40 years and older was similar to other age-estimation methods (average 36.9 years).

These patterns are distinct from those found combining TA3 traits using a transition analysis framework, which indicates issues with the analytical approach used, and not the TA3 trait reference data.<sup>3</sup> Most importantly, RGLM requires that individuals with missing data be removed from the reference sample to generate a training set that contains only individuals with all traits entered for the unknown case. This results in an inverse relationship between number of traits scored and size of the sample used to train the age-estimation model. Moreover, the demographic details of the training sample, which contains only a subset of the TA3 reference sample, are not available. Additional problems with the interface and source code were also identified; however, none were found to have significant effects on these results.

Based on these results, the current TA3 program is inappropriate for forensic or archaeological contexts and should be modified before stable release. As the TA3 traits have demonstrated potential to improve age estimation, additional analytical approaches that utilize the full TA3 reference dataset should be evaluated.

*Data collection was partially funded by National Science Foundation Doctoral Dissertation Research Improvement Grant (NSF DDRIG) Award #1455810 and an Forensic Sciences Foundation, Inc. (FSF) Field and Lucas Research Grant.*

### Reference(s):

1. Richardson, R.; Ousley, S.D. 2021. Transition Analysis 3 (version 0.8.0). <https://www.statemachine.net/software/TA3/>.
2. Milner, G.R.; Ousley, S.D.; Boldsen, J.L.; Getz, S.M.; Weise, S.; Tarp, P. 2019. *Transition Analysis 3 (TA3) Trait Manual*. Public Distribution Version 1.0. [https://www.statemachine.net/software/TA3/docs/TA3\\_Trait\\_Scoring\\_Manual\\_1.0.pdf](https://www.statemachine.net/software/TA3/docs/TA3_Trait_Scoring_Manual_1.0.pdf).
3. Getz, S.M.; Byrnes, P.D. 2021. Testing the Traits of TA3: Setting a Baseline for Method Development & Performance. Poster. *Proceedings of the American Academy of Forensic Sciences, 73<sup>rd</sup> Virtual Annual Scientific Meeting*. 2021. 27:55.

### TA3, Age-at-Death Estimation, Skeletal Biological Profile

## A50 A Test of Anthropological Accumulated Degree Day (ADD) Methods of Postmortem Interval Estimation on an Iowan Autopsy Sample

Natasha Andersen, University of Iowa, Iowa City, IA and Iowa Office of the State Medical Examiner, Ankeny, IA; Emily Archibald\*, Iowa Office of the State Medical Examiner, Ankeny, IA, and Valparaiso University, Valparaiso, IN; Taylor Brown, Iowa State University, Ames, IA and Iowa Office of the State Medical Examiner, Ankeny, IA; Hannah Spencer-Greathouse, BA, Iowa Office of the State Medical Examiner, Ankeny, IA and Simpson College, Indianola, IN; Jonathan Thompson MD, Iowa Office of the State Medical Examiner, Ankeny, IA; Heather Garvin, PhD, Des Moines University, Des Moines, IA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how two published anthropological methods of estimating postmortem interval from ADD perform on United States forensic cases in the state of Iowa.

**Impact Statement:** This presentation will impact the forensic science community by presenting accuracy rates for published methods, which can be used to assess method applicability in areas with similar climates and environments.

Megyesi et al. and Moffatt et al. are two of the most commonly utilized published methods for estimating postmortem interval in forensic anthropological cases.<sup>1,2</sup> The methods involve scoring the level of decomposition of three body regions (head and neck, trunk, and limbs) to develop an ADD estimate, then working backward from the date found to total up the average daily temperatures until the ADD estimate is reached, which is considered to be the point estimate for the date of death. Both methods also present 95% prediction intervals for estimates. Previous studies report variable results when applying these methods to cases or samples at decompositional facilities, with many expressing the need for region-specific formulae, given variability in climates and environments. This study aimed to test the accuracy of these two methods when applied to an Iowa forensic sample.

Seventy-one cases from the Iowa Office of the State Medical Examiner were identified for inclusion in the study. Only those cases in which decedents were found indoors (with recorded thermostat data) or outdoors on terrestrial surfaces were included. Signs of decomposition had to be evident and investigative documentation had to support the date of last contact as the likely date of death. The Megyesi and Moffatt methods were employed, scoring decomposition from scene and autopsy photographs, and using local national weather service temperature data to calculate ADDs. Accuracy of the methods was assessed based on percentage of cases that fell within the 95% prediction intervals and deviations between the method point estimates and presumed date of death.

Sample decomposition total body scores range from 4–30 (Megyesi) and documented postmortem intervals ranged from 0 to 255 days, although the sample is biased toward lower total body scores (Megyesi average=13, median=4) and shorter postmortem intervals (average=12 days, median=11 days). The Megyesi method is more accurate with 94.4% of the cases falling within the prediction interval as compared to 71.8% with the Moffatt method. The point estimates for the Moffatt method, however, are closer on average to the presumed date of death, with 10 cases with perfect point estimates and an average absolute difference of 13 days, as compared to 7 perfect point estimates and an average of 16 days for Megyesi. The Megyesi prediction intervals are significantly broader than the Moffatt intervals, thereby limiting utility, but the need for accuracy in forensic cases outweighs the desire for precision. Additional analyses are being performed to assess whether type/amount of clothing, sun exposure, environment, body size, or season of deposition can explain some of the variance observed.

### Reference(s):

1. Megyesi, M.; Nawrocki, S.; Haskell, N. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Sciences*. 2005;50(3):1-9.
2. Moffatt, C.; Simmons, T.; Lynch-Aird, J. An improved equation for TBS and ADD: Establishing a reliable postmortem interval framework for casework and experimental studies. *Journal of Forensic Sciences*. 2016;61(S1):S201-S207.

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### Forensic Anthropology, Time Since Death, Decomposition

## A51 Assessing Patterns of Moisture Content in Decomposing, Desiccated, and Mummified Tissue in the Southeastern United States: Trial 1 Data

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**Learning Overview:** After attending this presentation, attendees will understand patterns of desiccation observed in Western North Carolina between March 2021 and August 2021 and how these preliminary data compare to the findings of previous research.<sup>1</sup>

**Impact Statement:** This presentation will impact the scientific community by providing regional data of desiccation and mummification observed in an area that is classified through Köppen-Geiger as a warm-temperate, fully humid, warm summer (Cfb) region.<sup>2</sup> Additionally, this study provides a direct comparison to the study conducted by Lennartz and colleagues in central Texas in 2020.<sup>1</sup>

Medicolegal death investigators and forensic anthropologists are often asked to estimate the Postmortem Interval (PMI) of unknown remains. Accurately estimating PMI is a difficult task, particularly when remains are desiccated or mummified. Desiccation refers to the general moisture loss in tissue, while mummification is the point at which decomposition and decay are suspended.<sup>3</sup> Understanding the patterns of desiccation and mummification in different regions can inform PMI estimates and improve methods used to generate them. The goal of this study is to observe natural mummification, with particular attention paid to the sequence and rate at which it occurs in the southeastern region of the United States as compared to those in central Texas.<sup>1</sup> The research presented here originates from the first trial of the mummification study.

This project is occurring at Western Carolina University's (WCU) Forensic Osteology Research Station (FOREST). Three donors from WCU's Body Donation Program were enrolled in the first trial of this study (March 2021–August 2021). It was not possible to enroll additional donors as the research model stipulates donors be placed at a similar time due to weather and seasonality and there were no additional donors within this period to the Body Donation Program. Prior to placement, enrolled donors underwent an "intake" procedure where baseline moisture measurements, using the Delmhorst® RDM-3TM moisture meter with 22-E electrodes, and initial photos of the head, torso, and extremities were recorded. Twenty data points were collected for each donor daily, excluding weekends and inclement weather days, until all points were assessed as "inaccessible" due to skeletonization. A HOBO® remote weather station recorded hourly rain, temperature, solar radiation, and relative humidity. The data collected were graphically represented in a scatterplot that utilized Accumulated Degree Days (ADD) as a standardized time scale. In order to more accurately capture the curve of moisture loss, a Locally Weighted Scatterplot Smoothing (LOWESS) curve was added.

The relative moisture contents of the first trial individuals had conditional and marginal R<sup>2</sup> values ranging from 0.508–0.555, suggesting that 51–56% of the variation in moisture content was attributed to fixed (environmental) effects. Multi-level modeling of these fixed effects on each body region demonstrated that temperature has the greatest influence over moisture content with a fixed effects coefficient of -6.052, humidity and precipitation having less significant influence on moisture content with fixed effects coefficients of 0.635 and 0.275, respectively. Finally, solar radiation was not found to have a significant effect on moisture content, with a coefficient of 0.011.

The results of this trial correspond with those of Lennartz and colleagues in central Texas where the data demonstrated that environmental effects contributed to 50–55% of the variation in moisture content.<sup>1</sup> However, the data from this trial do not display an asymptotic relationship, which was observed in central Texas, and none of the donors in this study reached true mummification. The continuation of this study will examine if these trends persist or if seasonality impacts desiccation and mummification differently in the southeastern region of the United States. This preliminary data, however, do demonstrate that, despite regional differences in patterns of desiccation, there may be similarities in how environmental effects contribute to processes of mummification.

### Reference(s):

1. A. Lennartz; M.D. Hamilton; R. Weaver. Moisture Content in Decomposing, Deccesicated, and Mummified Human Tissue. *Forensic Anthropology* 2020; Vol. 3, No. 1: 1-16.
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### Postmortem Interval (PMI), Decomposition, Mummification

## A52 The Perimortem Trauma Pattern in Human Rights Violations: Comparative Case Studies From Spain, Cambodia, and Argentina

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**Learning Overview:** After attending this presentation, attendees will be informed about the potential of an anthropological approach to investigating mass graves from totalitarian regimes: Franco's Spain, Khmer Rouge, and Argentina's Guerra Sucia.

**Impact Statement:** This presentation will impact the forensic science community by reviewing how the scientific excavation of mass graves may serve different purposes in the collective rebuilding processes in the aftermath of violent conflicts and the critical role that forensic disciplines play.

On a global basis, violence has been directed against the victims for various reasons and in different manners and degrees, mainly depending upon socio-cultural practices and political ideology.<sup>1</sup> The late half of the 20<sup>th</sup> century has been characterized by an increase of incidents of genocide taking place around the world. Only in 1948 did the United Nations Genocide Convention first defined genocide as “the intent to destroy, in whole or in part, a national, ethnical, racial or religious group, as such.”

Genocidal practices are often associated with mass graves to conceal the crime and prevent individual identification, and forensic anthropology, along with other disciplines, has played a key role in investigating the systematic violation of human rights during armed conflicts and genocide.<sup>2</sup> The excavation of mass graves can help in the aftermath of genocides by providing closure for surviving families and successfully prosecuting those responsible for the crime.

This work presents a comparative study of three cases from totalitarian regimes responsible for committing gross human rights violations: Franco's Spain (1936–39), Khmer Rouge (1975–79), and Argentina's Guerra Sucia (1976–83). The purpose was to consider whether a pattern could be discerned in relation to the traumatic mechanism recorded by the skeletons and the demographics data of the victims.

The sample consists of 2,875 individuals: 1,762 of the Spanish Civil War, 643 of the Khmer Rouge Regime, and 470 for Argentina. The majority of victims appear to be male, aged between 20–35 years old. Although only a few studies mention the type of trauma inflicted, thus creating a bias for the overview, the most frequent cause of death was due to projectile trauma. The comparison between the three cases illustrates how dissimilar political beliefs can all be accomplices in unimaginable violence and crimes against humanity, despite the different contexts and manners in which they occurred. In addition, this research addresses some of the issues with finding scientific information and published literature on the matter.

The present research underlines the crucial contribution of forensic anthropology in analyzing cases of violation of human rights and mass grave investigations. The value of the discipline is represented by the exhumations and identifications that achieve reconciliation for the relatives of the victims, consequently accomplishing the search for truth and justice. The contribution has been quite evident in the cases presented, whereas great awareness has been raised about the rights of victims and their families.<sup>3</sup>

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### Forensic Anthropology, Human Rights Violation, Trauma Analysis



## A53 The Differences in Fracture Healing Based on Fracture Classification in Young Children (Less Than 6 Years of Age)

Diana Messer PhD\*, Western Carolina University, Cullowhee, NC; Andrielle Finch, Western Carolina University, Cullowhee, NC

**Learning Overview:** After attending this presentation, attendees will recognize differences in fracture healing between humeral fracture types in young children.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of the effect of fracture type on healing in young children and discussing implications for time since injury estimation in cases of suspected abuse.

Young children are most vulnerable to death resulting from physical abuse.<sup>1</sup> Certain fracture types and locations, such as humerus fractures without a known mechanism of injury and that are not immediately treated, are concerning for abuse.<sup>2</sup> Identification of such abuse may rely on accurate time since injury estimation of the healing fracture. Despite the influence of multiple factors on the healing process, including fracture type and fracture location, timelines of fracture healing are often applied universally.<sup>3</sup> The objective of this preliminary study was to examine the effect of fracture type on fracture healing in a sample of young children.

This retrospective review examined children five years old or younger radiographically evaluated for an unintentional humeral fracture at a pediatric level I trauma center between 2000–2016. Exclusion criteria included: (1) obscured casted radiographic exams, (2) unknown injury date, (3) individuals with bone disorders, and/or (4) fractures requiring instrumentation. Features of fracture healing (Subperiosteal New Bone Formation [SPNBF] and callus formation) were evaluated from initial and follow-up radiographic exams. Determination of fracture type was made by a medical professional and reported as written in the medical record. Analysis of fracture type was limited to the three most common within the dataset: transverse, spiral, and oblique.

Seventy-two humeral fractures were analyzed from 72 children (males: 35, females: 37). Of these, 41.6% (30) were infants one year or younger. A total of 232 radiographic exams were analyzed, with an average of 3 post-injury exams per child, and an exam range of 0 to 92 days (mean: 19 days); 93.1% (67) of fractures were complete and 55.6% (40) were diaphyseal. More than half the fractures were classified as transverse (58.3%, 42).

Across all fracture types, SPNBF and callus was present by three weeks post-injury. Analyses of Covariance (ANCOVAs) were performed to determine the effect of fracture type on fracture healing, controlling for child age. Post hoc analyses were performed with a Bonferroni adjustment. After adjustment for child age, there was no significant difference in first observation of SPNBF ( $p=0.955$ ). However, there was a statistically significant difference in first callus observation ( $p=0.052$ ), with oblique fractures observed earlier than transverse or spiral fractures (mean difference: 5 days). When all recorded observations were evaluated, there was no statistical difference based on callus type (soft  $p=0.914$ , intermediate  $p=0.132$ , hard  $p=0.841$ ). For intermediate and hard callus, there was a mean difference of between 5–6 days.

These results suggest that callus may form earlier in some fracture types than others. A five-day difference in appearance may have repercussions for identification of abuse in a forensic context or child protection in a clinical case. Increased sample sizes would improve statistical robusticity.

The results of this preliminary study may impact analysis and interpretation of healing fractures in cases of suspected abuse. Understanding the basic factors that influence fracture healing will ultimately lead to improved methods to estimate fracture age.

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### Fracture Healing, Child Abuse, Time Since Injury

## A54 Histological Differentiation of Burnt Cortical Bone in Northeast Ohio Mammals

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**Learning Overview:** Following this presentation, attendees will have a basic understanding of how burning affects the microscopic structures of mammalian cortical bone and whether species differentiation can be made using qualitative and quantitative methods.

**Impact Statement:** This presentation will impact the forensic science community by illustrating how varying temperatures impact the visibility of cortical bone microstructure in burned mammalian bone. Microstructural products of remodeling, such as secondary osteon size and shape, are essential for distinguishing human from non-human bone in fragmentary remains.

A first step in the analysis of skeletal remains of unknown origin includes determining whether the remains are human or non-human. Attempts to destroy a body or circumstances surrounding death involving fire often result in small fragments of bone, making it difficult to apply gross morphological techniques to identify species origin. Previous studies, including Dominguez and Crowder, have shown that histologic techniques can be used to identify whether a bone is human or non-human.<sup>1</sup> Dominguez and Crowder previously demonstrated that mean osteon area and mean osteon circularity can distinguish unburned human ribs, humeri, and femora from the same elements of deer and dogs with high accuracy. This study expands this methodology to burned skeletal remains to determine whether histomorphometry remains useful for distinguishing human and non-human remains at varying burn temperatures. This study hypothesized that: (1) aspects of osteon morphometry can be qualitatively identified in burnt skeletal remains exposed to varying temperatures, and (2) histomorphometric parameters such as osteon area and circularity can be used to quantitatively determine species origin of burnt skeletal remains from a selection of Northeast Ohio mammals.

The study sample consisted of right femora, ulnae, and sixth ribs from a variety of Northeast Ohio mammals (e.g., canine, deer, pig, cow) commonly encountered in forensic casework, and one human cadaveric femur. One-cm sections were procured from the diaphyses to be used as controls while the remainder were burnt in ceramic crucibles at 600°C, 800°C, and 1,000°C for 20 minutes using a muffle-furnace. All sections were subsequently embedded in methyl methacrylate using a protocol developed by the Andronowski Lab and ~150µm-thin sections were procured using a Well® Diamond Wire saw and mounted with Eukitt® mounting medium. Sections were visualized under brightfield at 100x using an Olympus® BX51 microscope, and overlapping images were acquired with cellSens® Entry software (v.1.16). Adjacent images were photomerged into a single image of the entire cross-section using Microsoft® Image Composite Editor (v.2.0.3.0) and Adobe® Photoshop® 2020. Morphometric analyses were performed in ImageJ (v.1.53k).

Preliminary qualitative results indicated that typical cortical histomorphometry is maintained at 600°C, including primary and secondary osteons, cement lines, osteocyte lacunae, vascular pores (Haversian canals), and cross-sectional geometry. The 600°C specimens revealed darker and more defined cement lines compared to controls. Collagen fiber orientation is also visible in 600°C specimens in primary and secondary bone under linearly polarized light, although not to the degree typical of unburned bone. This tissue histomorphometry is obliterated at 1,000°C, however, leaving only the spaces that characterize vascular pores and cross-sectional geometry as viable variables. This suggests a threshold of burn temperature for applying the Dominguez and Crowder method for distinguishing human and non-human remains, which requires distinguishable secondary osteons.

Results of this work potentially provide a new method of species determination in burnt bone, leading to the identification of fragmented burnt bone in a forensic context.

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### Histology, Forensic, Cortical Bone

## A55 Testing the Use of Bone Weathering to Estimate the Postmortem Interval (PMI) on Forensically Relevant Bone in an Arid Environment

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**Learning Overview:** After attending this presentation, attendees will be able to understand the limitations of using bone weathering to estimate PMI on human skeletal remains as well as understanding how complex environmental variables impact the temporal trajectory of bone weathering patterns.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that regional and microenvironments impact bone weathering patterns, making their use in the estimation of the PMI challenging.

Bone weathering is the process in which both the organic and inorganic elements of a bone separate and are destroyed by physical and chemical external factors *in situ*. Behrensmeier's seminal work developed a Likert scale for weathering patterns seen on bones, associating each stage with a PMI estimate.<sup>1</sup> This scale continues to be cited in recent research and textbooks as a measure for PMI in forensic cases.<sup>2-4</sup> However, observations at Colorado Mesa University's Forensic Investigation Research Station (FIRS) in Whitewater, CO, suggested that, in that environment, bone weathering rates may not follow the published literature.

The FIRS is a human decomposition taphonomic facility located in western Colorado, on the eastern portion of the Colorado Plateau. The facility is situated at an altitude of 1,457m Above Mean Sea Level (AMSL) in a semiarid steppe environment with relatively high solar radiation. The area generally receives less than 24cm of rain, and approximately 19cm of snow per annum. Remains generally desiccate and tissue may remain for years.<sup>5</sup>

To examine the relationship between bone weathering and PMI, a stratified convenience sample of 24 skeletons was selected based on PMI from the donated human skeletal collection at the FIRS. The PMI of the selected individuals ranged from 270 days to 1,768 days. Each skeletal element was scored using Behrensmeier's stages and descriptions.<sup>1</sup> Smaller bones in the hands and feet were excluded as per Behrensmeier.<sup>1</sup> Impacts to the bone from pathology and trauma were characterized and those skeletal elements excluded from data. A score for each skeleton was determined using the criteria of most advanced stage covering more than 1cm<sup>2</sup>.<sup>1</sup> It was hypothesized that solar radiation may impact bone weathering as others have noted sun bleaching and solar radiation as causes of accelerated bone weathering.<sup>3-4</sup> Hourly solar radiation levels measured from an on-site weather station were used to calculate Accumulated Solar Radiation Days (ASRD). Both PMI and ASRD were compared with the maximum weathering scores. The sample exhibited stages 0-4 of Behrensmeier's stages. Stage 5 was not present in the sample. The results were analyzed with a linear regression model and chi square test of independence.

In this sample, both PMI and ASRD have only a weak correlation with the maximum weathering scores. The data suggest that there is no significant association between PMI or ASRD and bone weathering, indicating that the amount of bone weathering is not related to either variable in this environment. Behrensmeier's stages of bone weathering, although excellent in the descriptions of bone weathering stages, should not be used for estimation of PMI in forensic cases as environment and other factors impact the trajectory of bone weathering.

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### Taphonomy, Bone Weathering, Postmortem Interval (PMI)

## A56 Serrated Versus Non-Serrated Blade Class Interpretation: An Interactive Survey

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**Learning Overview:** The goal of this presentation is to assess forensic practitioners' familiarity with blade class interpretation resulting from Sharp Force Trauma (SFT), and to collect survey data to establish baseline factors that may influence the accuracy of these analyses.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the challenges associated with blade class interpretation and demonstrating how blade characteristics influence the appearance of striation patterns on cartilage.

SFT analysis and interpretation have been extensively studied, particularly saw marks and blade kerf characteristics, and are recognized as a valuable tool in the forensic anthropology toolkit. However, the current knowledge base for adequately and accurately conducting such analyses remains insufficient. The variation in striation patterns imparted by non-serrated blades is largely unknown, potentially resulting in a misidentified or indeterminate blade class interpretation. This gap in knowledge is primarily based on the medium used for research. Most SFT studies use bone as a testing medium, limiting the appreciation of the range of variation in striation pattern produced by blades. Cartilage, in contrast, is a firm but compliant tissue with the potential to record a level of blade detail that bone cannot. Non-serrated blades may be coarsely milled and/or have defects along the cutting edge that are observable on cartilage but not bone.

Few studies have examined blade kerf characteristics using cartilage as a testing medium. A case study by Rao and Hart described the identification of a suspected blade by comparing the victim's costal cartilage kerf striation patterns to casts of the blade and test kerfs made in porcine costal cartilage.<sup>1</sup> Pounder et al. evaluated differences in kerf striation patterns in non-human costal cartilage imparted by serrated and non-serrated blades, but primarily focused on patterns imparted by serrated blades.<sup>2</sup> Finer striations associated with blade edge imperfections were occasionally observed, but these differences were not presented photographically.<sup>2</sup>

The aforementioned studies suggest that striae imparted by serrated blades can be readily differentiated from striae imparted by non-serrated blades. Kerf walls with a pattern of coarse striae or a mixture of coarse and fine striae that appear regularly spaced are generally interpreted as consistent with serrated blades (i.e., the serrated teeth leave coarse striations), and kerf walls with fine striae as consistent with non-serrated blades.<sup>3,4</sup> However, the observed striation pattern may not definitively indicate either blade class resulting in an indeterminate blade class interpretation. Furthermore, studies examining the reliability of blade class estimation from kerf characteristics suggest there is considerable potential for misidentifying blade class based on kerf characteristics.<sup>4,5</sup> Additionally, in this study's SFT case analyses (20–30 cases per year), distinguishing between serrated and non-serrated blades was found to be complicated by an array of factors, including blade quality and manufacturing variation.

This interactive presentation will assess the accuracy of blade class interpretation to generate baseline data regarding SFT analysis. Attendees of this presentation—primarily targeting forensic anthropologists and pathologists—will be encouraged to participate in an anonymous survey, accessible via Quick Release (QR) code, to evaluate their familiarity with blade class interpretation. Microscopic images of striation patterns on cartilaginous kerf walls and their associated casts (AccuTrans® casting medium) from six SFT cases analyzed at the Harris County Institute of Forensic Sciences will be provided to attendees to elicit interpretations. Blade class (i.e., serrated or non-serrated) for these cases were determined via review of scene/law enforcement photographs. Survey participants will choose from the following interpretations for each case: serrated blade/blade portion, non-serrated blade/blade portion, or indeterminate. A free text box will be associated with each response should the participant wish to provide an explanation. The following non-identifiable data will also be collected: (1) participant's forensic discipline/field; (2) education level; and (3) total number of cases in which the participant has evaluated blade class characteristics. These data will be used to ascertain what additional research and/or training is needed by forensic anthropologists, and possibly pathologists, to improve the accuracy and reliability of blade class interpretation.

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### Forensic Anthropology, Blade Class Interpretation, Striation Pattern

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## A57 The Usefulness of Transmission Electron Microscopy (TEM) for the Estimation of the Postmortem Interval (PMI): An Analysis of Ultrastructural Autolytic Changes in the Osteocyte of Human Remains

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**Learning Overview:** After attending this presentation, attendees will know an innovative use of TEM for the estimation of PMI.

**Impact Statement:** The ultrastructural study of the postmortem modifications of the osteocyte, by TEM use, could provide useful results for the estimation of the PMI in the first three months from death.

The estimation of the PMI of human skeletal remains is one of the most important issues in forensic sciences. Although further analyses have been performed on human remains, the PMI evaluation can be very changeable and difficult, especially in decomposed or skeletonized remains. Therefore, it should be very important to investigate the progress of bone diagenesis. This could be possible through the ultrastructural study of the autolytic modifications of the osteocyte by techniques not widely used in forensic pathology, such as TEM.

In this work, fragments of I rib were taken from eight subjects, males, aged between 25 and 50 years, without metabolic pathologies, subjected to autopsy. Each fragment was placed in an empty box, then placed in a laboratory with steady temperature and humidity. From each sample, at certain time intervals (PMI: 24h, PMI: 1 month, PMI: 2 months; PMI: 3 months; PMI: 4 months; PMI: 6 months; PMI: 10 months; PMI: 20 months), small fragments of cortical bone were taken. After specific preparation, inclusion in epoxy resin and microtome cutting with a diamond blade (90-120nm) were performed. The samples were observed at TEM, focusing on the ultrastructure of the osteocyte, detecting the progressive degeneration of nuclear and cytoplasmic components.

At PMI 0 it was possible to observe well-preserved osteocytes, with walls well attached to the osteocyte lacuna, the presence of a nucleus with normal chromatin, the evidence of preserved mitochondria and extensions containing cytoplasm; however, the first signs of cellular autolysis were evident: the dilation of the perinuclear cistern, the fragmentation of the endoplasmic reticulum, the presence of ribosomes clusters, the precipitation of the glycocalyx, the swelling of the mitochondria; at PMI 1, the core begins to detach itself from the gap; in some osteocytes the chromatin was thickened, typical of a pycnotic nucleus; in other osteocytes, cariorexes and caryolysis had already occurred; residues of endoplasmic reticulum and ribosomes were evident; at PMI 2, some osteocyte lacunae show large masses of cytoplasmic debris; from PMI 3 to PMI 20, the lacunae appear almost empty with peripheral cytoplasmic residues in the canaliculi.

The ultrastructural study of the postmortem modifications of the osteocyte, by TEM use, seems to provide useful results for the estimation of the PMI. In particular, appreciable differences were noted between PMI 0, PMI 1, and PMI 2, while from the third month after death, lacunae appear almost empty. This result makes it possible to use the bone matrix also for the estimation of PMI very close to the time of death, a case of great forensic interest.

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### TEM, PIM, Ultrastructure

## A58 The Effects of Thermal Alteration on Sharp Force Trauma in Human and Non-Human Skeletal Remains

Hailey Collord-Stalder, MA\*, Shasta County Coroner's Office, Redding, CA; Nicholas Herrmann, PhD, Texas State University, San Marcos, TX, USA

**Learning Overview:** After attending this presentation, attendees will better understand the morphological changes that occur from various Sharp Force Trauma (SFT) toolmarks in burned human and *Sus scrofa* skeletal remains.

**Impact Statement:** This topic will impact the forensic science community by addressing the ability to recognize peri- and postmortem SFT in thermally altered human and *Sus scrofa* remains and approximate the type of tool from the trauma.

Thermal alterations to human and Non-Human (NH) research analogs have been extensively documented in the literature, but fewer studies have addressed how tool class is identified from trauma in morphologically altered burned remains.<sup>1</sup> Additionally, though forensic investigators deal with human remains in their casework, experimental research has been relatively limited to use of NH proxies, which show substantial differences in bone morphology and composition. Questions addressed by this research are whether tool class can still be assessed from SFT in burned human and NH skeletal remains using digital microscopy and whether pigs are an acceptable NH proxy in toolmark research.

Data were collected on the long bone and axial elements of a human donor ( $n=19$ ) and one pig ( $n=41$ ). A total of 216 controlled toolmarks using a flat-edge knife blade, a serrated-edge knife blade, a reciprocating saw, and a handsaw were produced on each of the human and NH skeletal elements; thereafter, half were subjected to controlled thermal alterations. Kerf width, depth, length, angle, and the presence of striae were analyzed using a digital stereomicroscope for a total of 175 toolmarks. Profiles of each kerf mark were turned into 3D, manipulatable models.

Kruskal-Wallis tests indicated that there are significant differences with the flat-edge maximum width measurements among the four groups ( $H=8.737$ ,  $df=3$ ,  $p=.033$ ) and with reciprocating saw maximum width measurements among the four groups ( $H=10.868$ ,  $df=3$ ,  $p=.012$ ). Mann-Whitney U-tests further indicated where the significant differences arose.

A chi-square test of independence with a post-hoc Cramer's V test indicated that there is no statistical significance between the control and burned samples in presence of striae for the reciprocating saw, and that there is a weak correlation among the variables ( $\chi^2=0.865$ ,  $p=0.352$ , Cramer's  $V=0.136$ ). The results for the handsaw additionally exhibit that there is no statistical significance between the control and burned samples regarding presence of striae, and that there is a weak correlation among the variables ( $\chi^2=0.481$ ,  $p=0.488$ , Cramer's  $V=0.107$ ). The Goodness of Fit test results for the striae with the reciprocating saw depict statistical significance ( $\chi^2=26.064$ ,  $p<.001$ ). The results for striae with the handsaw also depict statistical significance ( $\chi^2=18.667$ ,  $p<.001$ ).

A goal of this research was to estimate tool class from trauma in burned remains and to assess the appropriateness of using pigs as NH analogs in experimental thermal alteration and trauma research. The results indicated that tool class was still identifiable in the burned remains and there were no significant differences between the representations and morphological changes in the human and pig remains. Future research is needed to diversify the sample and shore up the limitations of the trauma-infliction method, loss of data due to destructive analyses and preparation of samples, and the loss of natural biomechanical differences during trauma infliction.

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### Forensic Anthropology, Thermal Alteration, Sharp Force Trauma

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## A59 Wounded to Death: A Paleo-Forensic Analysis of a Case of Multiple Sharp-Force Lesions From the Medieval Cemetery of San Biagio in Cittiglio in Northern Italy

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**Learning Overview:** Through this presentation, attendees will have a practical example of how legal medicine and physical anthropology can interface in the paleo-forensic resolution of past violent events.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of ancient violence and showing how new interpretative and accurate data can be obtained through the analysis of skeletal remains.

The experimentation and development of innovative approaches, aimed at reconstructing the dynamics of ancient violent episodes, could have a historic-archaeological and forensic relevance. Without prejudice to the ethical standards of treatment of human remains, thanks to the “non-urgency” of the resolution of the case, it is possible to dedicate an in-depth and accurate study, approaching with different innovative tools that, once tested, may even be useful in the more crucial field of legal medicine.

Paleoforensic and paleotraumatology investigations constitute a significant scientific channel able to trace the history and the spread of violence in the past. Human remains from archaeological contexts occasionally provide traces of traumatic injuries, shedding light on the degree of interpersonal violence of a population, on the weaponry and technologies available at that time, and on the occurrence of certain historic events. Today, thanks to the advancement of scientific technologies, we can fully understand the extent of a lesion in order to shed light on the occurrence of the trauma.

As the most vulnerable and targeted area of the body, the skull often retains clear signs of the wounding weapon. Among all the types of injury, sharp-force lesions are frequently found on the skeleton of victims of violence and are bearers of significant information on the causative implement and modalities of wound formation.

This study describes a case of traumatic injuries recorded in a skeleton recovered during archaeological excavations at the medieval cemetery of San Biagio in Cittiglio (Varese, northern Italy). Evidence of multiple sharp-force trauma was observed on the skull, representing a unique case of raw violence in the past of this rural population.

The analysis of multiple lesions displayed on the skull required a systematic multi-analytical approach, combining classic macroscopic examination, computer tomography, photographic 3D modeling, and 3D digital microscopy in order to study the morphological and morphometric features of the lesions, verify the nature of the injuries, the direction of infliction to the bone, and the kind of weapon used. In particular, digital microscopic observation was performed in order to build magnified 3D images of the surfaces, examine the micro-morphological features, and conduct thorough qualitative and quantitative analyses.

Through the systematic study of the lesions, from the macroscopic to the microscopic level, this study was able to understand the dynamics of each lesion and hypothesize the reconstructive sequence of the violent event that led to the death of the victim.

In this study, the application of digital microscopy proved to be a necessary and non-replaceable technical tool for the analysis of microscopic marks on bones since it enables the analysis of bone surface changes by reconstructing a virtual model of their microstructure, whose features can be quantified and compared, enhancing the interpretation of the osteoarchaeological evidence.

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**Osteoarchaeology, Perimortem Trauma, Medieval Violence**

## A60 Decomposition in the Desert: Testing the Validity of Using Accumulated Degree Days (ADD) and Total Body Scores (TBS) for Postmortem Interval Estimation in the Sonoran Desert

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**Learning Overview:** After attending this presentation, attendees will understand the discrepancies in the application and utility of the ADD and TBS methods to postmortem interval estimation in a desert environment.

**Impact Statement:** This presentation will demonstrate that further geographically nuanced research in decomposition is critical to the development of a method for accurate, reliable estimates of postmortem interval.

The Sonoran Desert, which extends through southwestern North America and northern Mexico, presents unique environmental challenges both to sustaining life and to interpreting circumstances of death, particularly to assessing stages of decomposition and estimating the postmortem interval. Within a single month during the summer, temperatures have been documented to range between 58° and 124°F, with relative humidity as low as 7%.<sup>1</sup> In Arizona, this extreme climate is met with two growing populations who present a higher risk of heat-related deaths.<sup>2</sup> Maricopa County is part of the Tucson Sector of the United States-Mexico border, where more than 200 sets of remains belonging to known undocumented border crossers have been recovered since 1980.<sup>3</sup> The city of Phoenix also has a homelessness crisis, with 25,832 reported homeless individuals in 2015.<sup>4</sup> The vulnerability of these groups is further compounded by an increased likelihood for a longer interval between death and the recovery of the remains. An accurate postmortem interval estimate is critically important to the identification of deceased individuals in this region, thus the need to improve understanding of the progression and timing of decomposition in this environment is crucial.

The ADD method has been proposed as a means to estimate postmortem interval in forensic cases.<sup>5</sup> This method incorporates scoring the visual stages of decomposition through TBS with daily weather data to produce a postmortem interval range. Since its publication, the method continues to be applied and validated, and attempts have been made to revise the method to make it more accurate and precise.

In this study, ADD was estimated using two adult porcine models (*Sus scrofa domesticus*). The models were placed in an area of natural desert near Phoenix, AZ, and allowed to decompose through the month of June. One model was placed in direct sunlight and the other in the shade of a tree. Photographs were taken at 30-minute intervals, and temperature and humidity readings were taken at 10-minute intervals throughout the study. TBS was scored using photographs each day for both specimens. On day 8, the TBS for the shade specimen was 21, and on day 7 the TBS for the sun specimen was 17. The TBS did not change for either specimen after this point in the study. Using the formula provided in the original ADD method, these scores represent 95% prediction intervals for a postmortem interval of 1-38 days and 1-30 days for the shade and sun specimens, respectively. These ranges are too broad to be applicable to a forensic case and are concerning with regard to the same postmortem interval estimation being presented at one week versus after 25 days. The ranges are also counter to expectations based on visual observations of decomposition, as the shade specimen progressed through the visual stages of decomposition more rapidly, but presented a higher TBS and postmortem interval range. For this study, the ADD method was not precise enough to provide a reliable postmortem interval range and did not accurately reflect the nuances of decomposition in this region, even in a period of intense macroscopic changes. Further studies are needed in this environment to better ascertain the relationship between the numerous variables that influence decomposition, the process of decomposition, and postmortem interval estimation.

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### Accumulated Degree Days, Postmortem Interval (PMI), Decomposition



## A61 The Evaluation of Postmortem Interval (PMI) Using Collagenous to Non-Collagenous Ratios in Skeletal Remains: A Validation Study

Theresa De Cree, BA, Mercyhurst University, Erie, PA; Luis Cabo, MS, Mercyhurst University, Erie, PA

**Learning Overview:** After attending this presentation, attendees will better understand the applicability of collagen content measurements in determining the PMI.

**Impact Statement:** This presentation will impact the forensic science community by furthering research on determining time since death in forensic investigations.

Forensic anthropologists are called to assist in death investigations when remains are fully skeletonized or in an advanced stage of decomposition. Soft tissue changes are well documented and help to establish the short PMI, within a range of hours to a few days.<sup>1</sup> However, PMI becomes more difficult to establish as soft tissue becomes degraded or even absent. PMI estimation methods can utilize both intrinsic and extrinsic markers. For example, several estimation methods rely on external taphonomic agents and processes, such as insect colonization, while others rely on the intrinsic natural degradation process of bodily tissues. Bone collagen may be the most important of those tissue components at advanced PMIs, when bone is the only tissue remaining.

In this study, 17 male individuals ranging from 40–94 years of age and known PMI were utilized to investigate if collagen degraded at a predictable rate, through the measurement of Collagen to Non-Collagenous ratios (Co/NCo) in histological preparations of bone. The documented intervals ranged from 2 to 13 years at the time of staining. Success with this method was reported in Jellinghaus et al. on a sample of both modern and historical remains, the latter comprising PMIs outside the accepted timeline for forensic significance.<sup>2</sup> This study assessed the method on a larger, forensic sample.

Femoral samples were taken from each individual, thin sectioned, and stained with the Sirius Red fast Green Staining system from Chondrex.<sup>3</sup> This is an easily accessible method that can be applied with and without microscopy. In this study, microscopy was utilized. Microscope images of the stained bone samples were analyzed for red- and green-stained relative area ratios in MATLAB®.<sup>4</sup> Results revealed an apparent slight downward trend in the correlation between collagen ratios and PMI, but the relationship is not significant ( $P=0.653$ ). A paired *t*-test was performed to compare the results of this study with those expected from the equations reported in Jellinghaus et al., finding significant differences ( $P < 0.001$ ).<sup>2</sup> The majority of the sample for this study was part of the donated body program at Mercyhurst University. In that program, individuals are macerated and bones are stored in the collection. During the maceration process, the tissue endures temperatures up to 400°F. Thus, the results could be explained by the denaturation of collagen at these temperatures during processing.<sup>5</sup> This indicates that the method could not be applicable after standard bone processing. However, since no samples were stained completely green, if denaturation did occur, it was not total. Further research is required in this avenue.

The results showed a non-isometric relationship between the two parts of the utilized ratio, which would render the ratio transformation inappropriate for regression models such as the ones utilized by Jellinghaus et al. to develop their predictive equations.<sup>2</sup> Further studies utilizing unprocessed bone samples are necessary to confirm or reject this possibility.

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### PMI, Collagen, Bone Diagenesis

## A62 Differences in Fracture Types, Locations, and Severity in Pre-, Peri-, and Post-Menopausal Women.

*Danielle Delelio, BS\*, University of Nevada- Reno, Reno, NV; Kyra Stull, PhD, University of Nevada- Reno, Reno, NV; Laura Knight, MD, University of Nevada-Reno, Reno, NV and Washoe County Regional Medical Examiners Office, Reno, NV*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how the expression and location of blunt trauma varies throughout adulthood in females and its potential relationship to hormonal changes.

**Impact Statement:** This presentation will impact the forensic science community by creating a foundation for understanding how traumatic injuries vary through the female lifespan, which can inform practitioners by facilitating the interpretation of traumatic injuries.

Trauma analysis and interpretation is a key component for forensic anthropologists and pathologists during any analysis of remains. A recent study has suggested that frequency of fracture increases with age, and middle-aged and older females presented with the highest frequencies per age and sex group.<sup>1</sup> However, there is still a lack of empirical data on how intrinsic factors, including age and its associated hormonal changes, influence the appearance and severity of fractures. Hormones, such as estrogen, are present in substantial amounts during the pre-menopausal period but substantially diminish once a woman enters menopause. Estrogens work in conjunction with osteoblasts, hence a decrease in one will initiate a decrease in the other. Thinning of trabecular bone occurs as a result of decreased osteoblastic activity, and this is reflected in a low Bone Mineral Density (BMD). With a decrease in BMD related to age, we expect to see the number of fractures increase, but also the severity of fractures increase, and the location of fractures to change compared to pre-menopausal women.

The sample was collected from the Reno Orthopedic Center and the Washoe County Regional Medical Examiner's Office and included women older than 20 years of age with blunt trauma. Each of the 212 fractures were evaluated by type of fracture (comminuted, transverse, spiral, butterfly, oblique, buckle, avulsion, segmental, and longitudinal), location of fracture per bone (tibia, fibula, femur, radius, ulna, and humerus), severity of fracture (measured as displacement in centimeters), and completeness of fracture (25%, 50%, 75%, 100% complete). The sample was separated into three age groups relating to their presumed menopause status; individuals ages 20 to 49 years were classified as pre-menopausal ( $n=67$ ), individuals aged 50 to 55 years were classified as peri-menopausal ( $n=22$ ), and individuals aged 56 to 80 years were classified as post-menopausal ( $n=123$ ).

A chi-squared test of independence was conducted to reveal if different patterns existed between the age groups and specified covariates. Statistically significant results were found in all comparisons, except when bone had exhibited a fracture. The analyses revealed post-menopausal women suffered not only more frequent displacements, but also a greater severity in displacement ( $p=0.024$ ). Interestingly, most post-menopausal women had significantly different ( $p < 0.05$ ) fracture locations compared to younger women; specifically, post-menopausal women had a greater number of fractures located distally rather than proximal. The type of fracture was also statistically different across the lifespan ( $p=0.005$ ), with post-menopausal women sustaining more than double the amount of comminuted, oblique, and transverse fractures compared to spiral, avulsion, butterfly, longitudinal, and segmental fractures.

By using a large cross-sectional sample of women, we can reveal how the expression of traumatic injuries change throughout the female lifespan beyond the expectation of a higher likelihood of sustaining fractures. Documentation of trauma patterns elucidates normal and abnormal patterns in fracture type, location, and severity across the female lifespan and facilitates trauma interpretation for the forensic scientists. Continuation of this research, including a larger sample of peri-menopausal women, will provide a deeper understanding of the complex relationship between the skeleton, biomechanical forces, and the expression of injuries, allowing scientists to substantiate trauma interpretations and move beyond descriptive interpretations.

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### Menopause, Trauma Analysis, Blunt Trauma

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## A63 Fractur3D: A Computed Tomography Reference Atlas of Skeletal Trauma

*Terrie Simmons-Ehrhardt, MA\*, Richmond, VA; Sara Sochor, MS, University of Virginia, Charlottesville, VA; Anthony Falsetti, PhD, George Mason University, Fairfax, VA*

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**Learning Overview:** After attending this presentation, attendees will have learned what types of data can be extracted from computed Tomography (CT) scans containing skeletal trauma and embedded in an interactive web-based reference atlas to support forensic anthropological casework. Attendees will interact with examples and have the opportunity to provide feedback on features.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the advantages of interdisciplinary collaborations and the applicability of data sources generated outside forensic science to developing innovative and interactive reference databases utilizing modern web technology.

CT databases and the increased application of postmortem CT have expanded the scope of forensic anthropology methods to include virtual examination of skeletal features. Postmortem CT allows for the virtual examination of diagnostic skeletal features as well as skeletal trauma before removal of soft tissue for traditional forensic anthropological analyses. Virtual skeletal data from CT volumes can be viewed as 2D image sequences along any plane, as 3D volume renderings of soft and/or bone tissue, as 3D surface models, or any superimposed combination of the above, allowing for a comprehensive visualization and examination of skeletal injuries, including fracture lines, internal and external surfaces of affected elements, displacement and relative position of fragments, and trajectory/direction of injuries. This digital data can be used to build more comprehensive reference atlases of skeletal trauma to support forensic anthropology casework. In individuals with documented antemortem and/or perimortem skeletal injuries, postmortem or even post-autopsy CT scans can contribute to accessible and interactive references for forensic anthropologists to compare to injuries encountered in casework. Since not all practitioners should be expected to learn CT modeling and visualization methods, virtual skeletal reference data must be presented in accessible formats to prove useful.

The University of Virginia Center for Applied Biomechanics (UVA CAB) is recognized as one of the world's leading research groups in the field, specializing in impact biomechanics for injury prevention by using state-of-the-art equipment to analyze the intricacies of how the human body responds to injury. The Center's mission is to improve the quality of life through the mitigation and prevention of injury made possible by better understanding the mechanical response of the human body utilizing cadaveric donors. For many donors, pre- and post-experimental CT scans were collected, providing an opportunity to visualize superimposed skeletal elements before and after injury. Although UVA CAB does not perform weapon or ballistic experiments, several donors were identified with perimortem injuries such as gunshot wounds that were captured in CT scans, providing for additional trauma examples relevant to forensic anthropology.

A preliminary dataset of CT scans from UVA CAB were selected to populate a web-based 3D skeletal trauma reference database, Fractur3D, to be hosted at George Mason University. CT scans were collected with 0.625mm image spacing and converted to de-identified volumes with 3D Slicer. The primary advantage of using CT data for a skeletal trauma atlas is the ability to visualize internal surfaces and structures to better visualize the full extent and pathways of injuries. To make this data accessible to forensic practitioners, a web format using open-source javascript libraries that support 3D interaction with biomedical imaging data, such as transparency, clipping, and measurements, was implemented. This presentation will demonstrate the types of interactive data that can be extracted from CT scans and embedded in web pages so that other researchers may implement similar atlases. Web pages embedded with volumetric or 3D CT data provide a more modern, accessible approach to skeletal atlases beyond traditional photographic atlases.

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### Skeletal Trauma, Computed Tomography (CT), Fractur3D

## A64 The Effects of Soft Tissue on the Crystallographic Changes to Bone Mineral Upon Heating

Hannah Cross, MS\*, Keele University, Newcastle-under-Lyme, Staffordshire, United Kingdom; Charlene Greenwood, PhD, Keele University, Newcastle-under-Lyme, Staffordshire, United Kingdom

**Learning Overview:** After attending this presentation, attendees will better understand the effects that heating bone in conjunction with soft tissue has on bone specimens.

**Impact Statement:** Nearly all heated bone studies have utilized dry, defleshed bone. However, the results of this study have highlighted differences between fleshed and defleshed bone upon heating, which must be taken into consideration when applying such techniques to forensic casework.

Upon the recovery of burnt remains in a forensic or archaeological context, bone is often fragmented and commingled, making differentiation between human and non-human samples extremely challenging and subjective. Due to thermal degradation of the organic component, biological techniques, such as DNA analysis, are often rendered futile, so attention is drawn to the final surviving component of bone, the mineral hydroxyapatite. Exploring the physicochemical modifications that occurs to hydroxyapatite upon heating has shown promise in differentiating between species based on characteristic changes within its crystal lattice structure and the presence of extraneous mineral phases.<sup>1</sup> However, the effects soft tissue has on the heat-induced changes are not fully understood, yet are of paramount importance as most bodies are intact, not skeletonized, during a burning event. This study aims to explore the effect heating has on fleshed bone, specifically investigating modifications to the nanocrystalline structure of bone mineral, and whether this has a significant impact on species differentiation.

Femoral cortical bone was taken from bovine specimens. Varying weights (5g, 7g, and 10g) of muscle and fat, and one layer of skin (10g) were tested separately to understand their individual affect. A combination of the three tissue types was also considered. The samples were heated for two hours at various temperatures (200°C, 400°C, 600°C, and 900°C). Powder X-Ray Diffraction (pXRD) analysis was utilized to calculate coherence length and lattice parameter values and the weight percentages of extraneous mineral phases formed during the thermal degradation of hydroxyapatite. Coherence length, which gives an indication on crystallite size and strain, was calculated using the Scherrer equation and the Full Width Half Maximum (FWHM) peak values. Fourier Transform Infrared (FTIR) spectroscopy was utilized to collaborate XRD data and to further our understanding of the relationship between the degradation of the organic matrix and the crystallographic changes. The color changes to bone were also measured using a Colorimeter.

It has been demonstrated that overall, soft tissue has a shielding effect on bone, reducing the expected thermally induced modifications. This is most prominently observed in samples heated with a combination of muscle, fat, and skin, which is likely due to the larger quantities of tissue protecting the sample from the thermal energy required to induce physicochemical modifications. Significant differences ( $p < 0.05$ ) were observed between defleshed samples and those heated with a combination of tissue types with at least one tested parameter when heated at 400°C, 600°C, and 900°C. Furthermore, the crystallographic parameters of defleshed samples were found to be significantly different ( $p < 0.05$ ) to all other fleshed sample types when heated at 900°C. Therefore, the effects of soft tissue must be considered when applying findings to forensic and archaeological heated bone cases whereby samples have been heated at these higher temperatures. Further research must now be conducted that incorporates different species of bone, heated in conjunction with soft tissue, to determine whether species differentiation can still be achieved using such parameters.

### Reference(s):

<sup>1</sup>. Beckett, S.; Rogers, K.D.; Clement, J.G. (2011). Inter-Species variation in bone mineral behaviour upon heating. *J Forensic Sci*, 3, 571-579.

### Heated Bone, Fleshed Bone, Bone Nanostructure

## A65 Identifying Taphonomic Factors Affecting the Dispersal Patterns of Surface-Scattered Human Remains: A Study of a Forensic Sample From Actual Casework

Kristine Kortonic, BS\*, Mercyhurst University, Erie, PA; Luis Cabo-Perez, MS, Mercyhurst University, Erie, PA

**Learning Objectives:** Attendees of this presentation will gain a better understanding of the significance of specific taphonomic factors on the deposition patterns of surface-scattered human remains.

**Impact Statement:** This presentation will impact the forensic science community by presenting findings from a retrospective taphonomic study of a forensic anthropological sample with a wide range of postmortem intervals (PMI) and environmental conditions not previously studied.

An understanding of a wide variety of taphonomic factors, agents, and processes, and their effect on the final deposition of human remains at the outdoor scene is crucial to forensic taphonomic interpretation and crime scene reconstruction.<sup>1</sup> Although distribution patterns are commonly utilized in forensic taphonomic analysis to reconstruct the death event, there has been little applicable research into the subject.<sup>1</sup> At death scenes, investigators are tasked with answering questions regarding the PMI, scattering of skeletal elements, damage to remains, and absence of skeletal elements. These questions are addressed through thorough analysis of taphonomic data collected from the scene and the human remains. Analysis of the distribution patterns of the remains and associated evidence in relation to environmental features is used to understand the processes that lead to the final disposition of the remains and ultimately reconstruct the death event.

Research into the common dispersion patterns of surface-scattered human remains within the context of the scene can be used to identify the specific taphonomic variables and their effect on the final distribution patterns of human remains and associated evidence. In addition, quantifying the common distribution of surface-scattered remains would assist in structuring scene recoveries and training protocols. Many of the current studies on distribution patterns of surface-scattered remains have utilized small-sampled, non-human studies with short PMI. These studies are useful for identifying possible effects of different taphonomic factors, but the lack of actualistic studies on human models with extended PMI limits the applicability of the findings to forensic cases.

One retrospective study conducted spatial analysis on a sample 36 cases of surface-deposited human remains in Louisiana to investigate patterns in body dump location and dispersal overtime.<sup>2</sup> A significant correlation was found between PMI and the shortest dispersion distance, but no relationship was found between PMI and the greatest or average distance of dispersion. These findings contradicted the conclusions of a previous retrospective study with a maximum PMI of 4 years.<sup>3</sup> The latter study found that the recovery of skeletal elements decreased with increased PMI; indicating that remains became more dispersed over time.<sup>3</sup> Although studies have suggested that particular taphonomic variables such as clothing, scene topography, and vegetation density have specific effects on the distribution of surface-scattered human remains, the effect of these factors has not been directly studied.<sup>1</sup>

The goal of this study was to analyze the preservation and distribution patterns of human remains in a forensic anthropological sample in relation to specific taphonomic variables including PMI, topography, and animal scavenging. This study utilized a forensic sample of 41 forensic anthropological cases with a known PMI range of approximately 2.5 months to 22 years. Each case was recovered through forensic archaeological methods and had full forensic osteological and taphonomic analyses. Spatial data was collected for each case by assigning cartesian coordinates to each individual skeletal element from scene maps using image processing software. The spatial distribution of each case was analyzed in relation to the known PMI, topographic data collected from the scene, the skeletal inventory, and osteological evidence of animal scavenging. The relationship between the dispersion and recovery of skeletal elements, and taphonomic variables, including PMI, scene topography, and animal scavenging, has been investigated using statistical analysis.

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1. Dirkmaat, D.C. 2012. *A companion to forensic anthropology*. Sussex, UK: Wiley-Blackwell.
2. Manhein, M.H.; Listi, G.A.; Leitner, M. 2006. The application of geographic information systems and spatial analysis to assess dumped and subsequently scattered human remains. *Journal of Forensic Science*. 51(3).
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### Forensic Taphonomy, Postmortem Interval (PMI)SA, Forensic Archaeology

## A66 Taphonomic Change in the Sonoran Desert: Monitoring Morphology in the Period of Mummification/Advanced Skeletonization

Kassandra Parrish, BS\*; Arizona State University, Glendale, AZ; Professor Katelyn Bolhofner, PhD, Arizona State University, Glendale, AZ

**Learning Overview:** The major goal of this presentation is to utilize subaerial taphonomic data to better understand the morphological changes that occur in the process of natural mummification and advanced skeletonization in a desert environment.

**Impact Statement:** This presentation will impact the forensic science community by establishing expectations for the degree of morphological change that may be observed during late stages of decomposition in a hot and arid desert climate, with the ultimate goal of improving estimates of postmortem interval in this region.

The Sonoran Desert covers vast regions of Mexico, California, and Arizona. As with other desert environments, extreme temperature fluctuations are characteristic of the region. From May through September, average daily temperatures may range from 69°F to 120°F at the end of August. In contrast to other desert climates, though, a significant period of rainfall occurs during both the winter and summer seasons, resulting in a diverse range of vegetation and wildlife and producing a very unique climate overall. In Arizona, the summer “Monsoon Season” of June through September, coupled with the extreme heat at this time of year, produces a drastic wet/dry cycle, resulting in unique patterns of variability in the decomposition rates and stages of human remains and directly impacting estimates of postmortem interval.<sup>1</sup>

This unique climate and its impact on estimates of postmortem interval become a critical issue for medicolegal death investigators confronted with the challenge of addressing the deaths of undocumented border crossers in this region. Despite the extreme temperatures and intense storms during the summer in Arizona, crossings at the United States-Mexico border at this time of year can reach peak numbers with fatal results for many. In 2020, 227 migrants’ remains were found. In June of 2021 alone, 43 migrants’ remains were found along the border.<sup>2</sup> Often, remains are recovered in advanced stages of mummification and skeletonization, states in which postmortem intervals are particularly difficult to ascertain, further complicating identifications and death investigations.

To begin to address these issues, the goal of this study is to monitor visual changes associated with the late decomposition stages of mummification and advanced skeletonization. Here, this study presents the initial results of an ongoing, long-term study intended to track the timeline of changes through these stages. Two porcine models with minimal tissue adhering were placed in observation cages in a natural desert environment in Carefree, AZ, on May 22, 2021. One cage was placed in direct sunlight and the other was placed in the shade of a Palo Verde tree to assess whether sun exposure had a significant impact on the rate or stages of mummification or advanced skeletonization. Visual observations and digital images were recorded once a week for the first month and every other week to the present date.

The results of the initial phase of this project (to date: 4 months; 92 days) demonstrated rapid mummification within the first two weeks of initial placement and minimal skeletonization, followed by very little measurable change to date. Currently, both models appear mummified and little-to-no skeletal elements are exposed, leading to almost no observable change since initial placement. There has been minimal rodent activity and minimal insect activity observed.

Observations are ongoing, with the ultimate goal of developing a nuanced system of expected taphonomic change in this period of decomposition in a hot and arid environment, both to update existing systems and to provide data that may contribute to improved estimates of postmortem interval when remains are found in this condition.<sup>3</sup>

### Reference(s):

1. Fulp; K.J.; Gast, M.M.; Harris, J.A.; Bolhofner, K.L. Decomposition in the Sonoran Desert During the Summer. *Proceedings of the American Academy of Forensic Sciences, 73<sup>rd</sup> Virtual Annual Scientific Meeting*. 2021.
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### Taphonomy, Advanced Decomposition, Sonoran Desert

**A67 Identification of Differential Burning Among Highly Fragmented Cremated Human Remains**

Lisa Monetti, MSc\*, University College London Institute of Archaeology, London, United Kingdom and Drew University, Madison, NJ

**Learning Overview:** Attendees, after attending this presentation, can expect to better understand the potential for the use of image analysis software toward the identification of differential burning patterns among highly fragmented burned human remains. Attendees will learn that such analysis can contribute toward the reconstruction of burning scenarios for remains that would otherwise not reveal such information.

**Impact Statement:** This research will impact the forensic science community by presenting a free and accessible method for the interpretation of highly fragmented burned human remains. The method allows for feasibility toward the reconstruction of the burning scenario through the identification of subtle but significant differential burning, which was previously out of reach given fire's ability to obscure a great deal of information on burned bone. Instead of working against the heat-induced changes, this method approaches analysis by interpreting such changes.

The analysis of burned human skeletal remains requires specialized experience and expertise. In cases where remains are highly fragmented, such analysis is further complicated and far more time-consuming. Bones transform dramatically during heating, but many of the factors contributing to these heat-induced changes have been realized through experimentation and observation of forensic cases.<sup>1,2</sup> As a result, the identification of differential burning and unexpected burn patterns can aid the anthropologist in estimating and reconstructing burning scenarios. A particularly useful example is extreme changes in color representing areas that were differentially covered with flesh or other materials during burning, reducing the bone's exposure to heat in some areas, while allowing others to char.<sup>2</sup> The areas that were covered maintain a white color, while the exposed areas appear black from charring. This type of macroscopic observation of differential burning can indicate the direction of the fire's movement or the location of the body in relation to the heat source.

Differential burning on a macroscopic level is a reliable and valuable observation for the forensic anthropologist when reconstructing the burn scenario. Completely calcined remains, however, do not allow the forensic anthropologist to observe macroscopic differential burning, as the remains can appear uniform and are often highly fragmented. While calcined remains with high levels of fragmentation can also obscure elements of the biological profile, this presentation reveals an alternative avenue of analysis: the interpretation and estimation of the burning scenario through identification of differential burning on highly fragmented remains. This presentation explores the applicability of image analysis software toward the identification of differential burning across the human skeleton in cases where macroscopic observation is otherwise impossible.

Image analysis software allows the anthropologist to observe changes in the bone's morphology through shape descriptors that examine the overall shape and size, the shape of the object's corners, and the texture of the edge of the fragment. These represent large-, medium-, and small-scale analyses, respectively, which are not attainable through standard methods of observation. The morphology of the remains is directly linked to the burning process, as fragmentation is caused by heat-induced fractures.

This project examined 6,712 fragments of burned human remains using image analysis to calculate values representative of bone morphology, and heat-induced fractures were also quantified for each fragment. These data were analyzed using Non-metric Multidimensional Scaling (NMDS) to identify patterns of differential burning, and the results were compared with standard methods for identifying differential burning on the same fragments. The results of the NMDS and post hoc Permutational Multivariate Analysis Of Variance (PERMANOVA) indicate that differential burning can be observed using image analysis software to a significantly ( $p < 0.05$ ) greater degree than can be completed through standard anthropological analysis.

The future implications of this research are significant. Not only does it create new possibilities for the interpretation of a burning scenario, it also utilizes free image analysis software (ImageJ), making the analysis of burned human remains more accessible to smaller or underfunded labs or less-experienced practitioners. Further, more preliminary results of an ongoing follow-up study indicate that image analysis combined with Random Forest machine learning allows for rapid classification of remains into skeletal region and bone type based on the shape descriptors alone. This presentation explores the possibility of utilizing image analysis toward identifying differential burning patterns in highly fragmented burned remains and also identifies the value and future potential for this accessible method. Analysis of burned human remains is often a struggle against the heat-induced changes caused by fire, but this method allows those changes to instead contribute to their interpretation.

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**Differential Burning, Cremation, Image Analysis**

## A68 Tuning Microbial Succession-Based Postmortem Interval (PMI) Estimation Models: The Effect of Environmental Parameters on Model Prediction

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**Learning Overview:** After attending this presentation, attendees will better understand the application of random forest models to predict PMI from soil microbial succession during human decomposition. Specifically, this presentation will increase attendees' knowledge of how inclusion of environmental parameters (e.g., pH, conductivity) impact model accuracy.

**Impact Statement:** This presentation will impact the forensic science community by considering additional data in microbial-based models for the purpose of improving PMI estimation in a human system.

Establishing PMI, or time since death, is often pertinent to death investigations, providing information to establish a timeline of events.<sup>1</sup> Multiple methods for PMI estimation exist, including estimation via insect ecology and morphology changes over time.<sup>2,3</sup> Recently, microbes have been suggested as potential predictors of PMI, as microbes are ubiquitous and change during decomposition<sup>4-6</sup>. A few models have attempted to assess the predictability of microbiome succession on the skin and in decomposition soils and estimate PMI.<sup>4,7</sup> These models generally include application of machine learning techniques to taxonomic marker genes (e.g., 16S rRNA, 18S rRNA) datasets and produce error ranges from 2.5 to 7 days.<sup>7</sup> While promising, these models are based solely on sequencing data and do not consider environmental factors. Therefore, the goal of this work was to evaluate how inclusion of environmental data in microbial-based models impacts PMI estimation from soil samples collected during human decomposition.

Research data were obtained from a 19-individual ("donors") decomposition study completed at the University of Tennessee Anthropology Research Facility (ARF) in Knoxville, TN. Soil samples (0-5cm) were collected from the decomposition-impacted area surrounding donors at predetermined Accumulated Degree Hours (ADH) intervals until the end of active decomposition. Soil samples were homogenized and either flash frozen for DNA extraction or used to measure soil pH, electrical conductivity, and extracellular enzyme activities; bacterial community composition was assessed using 16S rRNA gene amplicon sequencing.<sup>8,9</sup>

Random Forest regressors were applied to taxa abundance or taxa abundance plus soil data, respectively, to create PMI estimation models. Only samples  $\leq 5,000$  ADH were used to keep timepoints consistent between donors. Models were created using Operational Taxonomic Units (OTUs) and at three taxonomic levels (order, class, and phylum) in order to assess the effect of taxonomic level on model predictions. All models were generated in R using the Ranger package.<sup>10</sup> Model accuracy was measured by calculating the Mean Square Error (MSE) and Mean Absolute Error (MAE), then compared between models.

Across all models, MAE ranged from 804.3 ADH up to 884.5 ADH. Of the models without environmental data, the order level was most accurate (MAE 814.2), while all others performed similarly. Effect of environmental parameters on model accuracy differed based on taxonomic level. At the phylum and class levels, addition of environmental parameters improved MAE by 48.6 and 65.1, respectively. In contrast, models with and without environmental data performed similarly at the order level, while models at the OTU level were negatively affected by the inclusion of soil data. Overall, the phylum and class level models with environmental data resulted in the lowest MAE (805.0 and 804.3, respectively).

Within this dataset, the inclusion of environmental data generally improves model predictability, but this result is dependent on taxonomic level and sampling timeframe. Additionally, only a few environmental variables were considered, and others (e.g., nitrate, dissolved carbon, etc.) may improve models further and should be investigated. These preliminary results differ from those previously reported, suggesting the impact of different sampling conditions and size and data processing methods on model accuracy, and highlighting the need for large-scale validation of microbial-based PMI estimation models.<sup>7</sup>

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## **Microbial Succession, Postmortem Interval (PMI), Random Forest Model**

## A69 The Importance of Recognizing the Indicators of Vulture Scavenging for Forensic Archaeological Recoveries and Taphonomic Interpretations

*Kristine Kortonick, MS, Mercyhurst University, Department of Applied Forensic Sciences, Erie, PA; Anthony Lanfranchi, MS\*, Mercyhurst University, Department of Applied Forensic Sciences, Erie, PA; Luis Cabo-Perez, MS, Mercyhurst University, Department of Applied Forensic Sciences, Erie, PA*

**Learning Overview:** After attending this presentation, attendees will have an understanding of the importance of the utilization of forensic archaeological methods to support accurate forensic taphonomic analyses and outdoor crime scene reconstruction.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of forensic archaeological methods for a proper forensic taphonomic analysis utilizing a novelty forensic anthropological case of vulture scavenging of human remains.

Animal scavenging activity has the potential to alter significantly the final deposition of human remains and the surrounding environment. Thus, understanding the behavior of different scavenger species becomes crucial to detect and interpret their effects as taphonomic agents in forensic settings. Indeed, scavenging activity has been reported to affect Postmortem Interval (PMI) markers, the spatial patterning of surface-scattered remains, and postmortem bone preservation and alteration, thus, affecting PMI inferences, trauma interpretation, and scene reconstruction.<sup>1,2</sup> Vultures in particular are known to alter human remains and the site of deposition due to rapid consumption of soft tissue, dispersion and damage to the remains, and alteration of the contextual environment.<sup>1</sup> Therefore, recognizing the indications of animal scavenging associated with different types of scavengers can improve the interpretation of the scene.

Published data on the effects of animal scavenging in Pennsylvania are extremely scarce and limited to sporadic observations on a reduced set of scavenging species. Regional differences in scavenger behavior have been reported for an even more limited number of species. There are some episodic reports of migratory patterns of vultures in Pennsylvania, but a lack of published literature about their presence and behavior in Central and Western Pennsylvania.

The first comprehensively documented example of vulture scavenging activity on human remains in Central Pennsylvania, was the human remains representing one individual located in the woods in a state of advanced decomposition. The individual was almost completely skeletonized and was significantly scattered. Most of the lower limb was not recovered. The main concentration of material included clothing, a partial vertebral column, and multiple ribs. No clear decomposition stain could be identified. Twenty feathers consistent with those of a turkey vulture (*Cathartes aura*) were collected from the scene. Superficial scratches were visible on the materials covering the surface of some skeletal elements, but they washed away as the remains were cleaned for osteological examination. Additionally, extensive damage was present in the orbital areas, in particular on the lacrimals and ethmoid. The scene was comprehensively documented following forensic archaeology protocols. Despite the nearly skeletonized state of the remains, the individual had only been missing for less than four months. It has been suggested that approximately two years are required for the removal of all soft tissue by natural processes in Pennsylvania.<sup>2</sup> Thus, if the evidence of vulture scavenging had not been considered, the PMI would have been likely overestimated by around two years. The evidence of scavenger activity present at the scene provided the necessary information to detect and correct the bias introduced by vulture activity, resulting in a more accurate, scientifically backed taphonomic interpretation.

Aside from providing the first documented information on forensically-significant vulture scavenging in Central Pennsylvania, this example of animal scavenging illustrates the importance of proper recognition and collection of taphonomic evidence, and presents basic scene processing protocols and strategies to detect and interpret this type of information.

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### Scavenging, Forensic Archaeology, Taphonomy

## A70 The Bone Weathering Simulator: An Investigation of Bone Change Informing Forensic Anthropology Recovery Best Practices Using Crowd Sourcing

*Matthew Rolland, MS\*, Florida Gulf Coast University, Fort Myers, FL; Heather Walsh-Haney, PhD, Florida Gulf Coast University, Fort Myers, FL*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how to introduce Science, Technology, Engineering, and Math (STEM) students to the effect that geological, environmental, and human-induced movement has on bone preservation in addition to the utility of crowdsourcing data collection through virtual platforms for scientific investigation.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of taphonomic change to skeletal material “recovered” through forensic anthropological best practices.<sup>1</sup> Additionally, a novel research design and data collection methodology will be presented that may be extended for further scientific investigations.

As part of the Florida Gulf Coast University (FGCU) 2021 Whitaker Center’s Summer Research Opportunities (SRO), students evaluated changes to chicken (*Gallus gallus domesticus*) and Burmese python (*Python bivittatus*) bones in weathering simulators. In essence, the students simulated the passage of time and the effect that weather, substrate, and water (all postmortem or after-death changes) have on bone preservation by sifting the chicken and python bones through ½" and ¼" inch screening. The students evaluated how mechanical hand sifting contributes to the destruction of bones and how effectively the ½" and ¼" screening catches forensic evidence.

Each student received one bone weathering simulator (i.e., one 23 x 16 x 6 Sterilite container with lid, one soil sieve with ¼" screening, one soil sieve with ½" screening, five sterile chicken bones, and five sterile python bones) and one type of substrate (gardening soil, sand, pebbles, and rocks). Some students additionally received distilled water.

All the participants were split into groups of five or six and each group was defined by the substrate inside their bone weathering simulator. Each student placed a bone of each type in each sieve with the substrate. The students shook the substrate through the sieve until all of the substrate had worked its way out of the sift. Taphonomic changes to the bones at each level were recorded and photographed.<sup>2</sup> Due to COVID-19 contact restrictions, the students were led through their data collection operations at their own homes via Microsoft® Teams. This study argues that rather than a hindrance to the data collection procedure, this virtual crowdsourcing of data collection allowed larger volumes of reliable data to be gathered over a shorter time frame than traditional data collection methodologies. As such, crowdsourcing data collection has far-reaching implications for forensic research and is gaining traction in areas of scientific research other than forensic anthropology.<sup>3</sup>

This study additionally presents data that statistically significant taphonomic differences were observed between skeletal material sifted through the varying substrates and water conditions as investigated by R Studio General Linear Models ( $t(5)=.71, p=.018$ ).

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### Forensic Anthropology, Taphonomy, Crowd Sourcing

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## A71 World Trade Center—A Historical Overview

*Amy Mundorff, PhD\*, University of Tennessee, Knoxville, TN; Eric Bartelink, PhD, California State University-Chico, Chico, CA; Jason Wiersema, PhD, Harris County Institute of Forensic Sciences, Houston, TX; Gaille Mackinnon, MSc, Alecto Forensic Services, Watchfield, Oxfordshire, United Kingdom*

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**Learning Overview:** This presentation will provide an overview of the World Trade Center (WTC) attack and identification efforts. The goal of this presentation is to present a historical perspective of events in New York City on September 11, 2001, and how anthropological contributions developed following the large-scale loss of life. After attending this presentation, attendees will better understand the initial role of anthropology in the management of the WTC victim identification effort, in particular, the different mortuary approaches adapted to address extensive fragmentation and commingling, and how this effort paved the way for identification protocols in subsequent mass fatality incidents.

**Impact Statement:** This presentation will impact the forensic science community by discussing the challenges of an open-population mass fatality with an ongoing search operation and a complicated recovery site at a time when most disaster preparedness exercises were geared toward events no larger than a plane crash. This presentation will discuss the continuous evaluation and changing approach to mortuary work due to extreme commingling, calcined remains from hot spots burning for longer than three months, and the challenges of rapidly developing new DNA technology.

Ground Zero encompassed 16 acres and reached 140 feet in depth. Buried within the 1.7 tons of debris were fires exceeding 1,500°F and the remains of 2,749 deceased individuals. The site comprised multiple events as the North Tower collapse pushed through the debris of the collapsed South Tower. Within hours, the Office of Chief Medical Examiner (OCME) began implementing plans to identify the victims, issue death certificates, and facilitate returning remains to next of kin.

It became clear that lower Manhattan was not conducive to this scale of work, so plans were made to utilize the closed Fresh Kills Landfill on Staten Island, including the water barge system for direct transport of the debris. At the landfill site, building material was sorted from dirt, which was sent to massive sifting screens and on to conveyor belts for remains recovery. This facility was in operation 24/7 through to August 2002, three months after the closure of Ground Zero. More than 4,200 fragments of human remains were recovered from this operation.

Recovery operations at Ground Zero were complicated by prolonged search efforts for survivors and the need for large machinery to assist in deconstructing such a dangerous site. As decomposition set in, already fragmentary remains were further disrupted and commingled by grapplers and excavators. Human remains were collected by the Fire Department of the city of New York (FDNY) and transported to the OCME multiple times a day. However, due to lack of training in identifying and recovering compromised remains, additional incidents of commingling occurred during recovery. Further complicating identifications, the forces from the building collapses rendered remains so fragmentary that fingerprints and dental matching were limited to the few hundred that had teeth or fingers present. DNA would become increasingly important to identify the nearly 22,000 fragments recovered, of which more than 5,000 were no larger than an inch.

The unprecedented commingling was not initially evident; for example, tissue from one individual fused to bone of another, and bone fragments from multiple individuals were found embedded in others. As it became clear that instances of commingling may have been missed, two programs were implemented to add quality control measures to the identification efforts. The Anthropological Verification Project focused efforts on re-examining over 18,000 cases to look for potentially missed commingling. The Final Anthropological Review entailed examining identified remains as part of the reconciliation process, to ensure a final check of each identified fragment prior to approving its release. These two programs illuminated unforeseen issues, contributed to additional identifications, and ensured that initial protocols were continually assessed to respond to the unprecedented scale of this incident.

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### Commingling, Mass Disaster, WTC

## A72 The Role of Forensic Anthropology in the Aftermath of the 9/11 Attack on the Pentagon

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**Learning Overview:** This presentation will provide an overview of the methods, roles, and types of information that forensic anthropologists can contribute to the identification of victims of mass disasters.

**Impact Statement:** The 9/11 attack on the Pentagon was a tragic historical event that we in the forensic community should remember and learn from.

Events on the morning of September 11, 2001, would forever change the world. Terrorists had hijacked four commercial airplanes and flown them into the Twin Towers in New York City, the Pentagon in Virginia, and an open field in Shanksville, PA. American Airlines Flight 77 departed Dulles Airport near Washington, DC, at 8:20 a.m. en route to Los Angeles, CA. Five hijackers took control of the plane and crashed it into the Pentagon at 9:37 a.m., killing all 64 passengers and crew and 125 military personnel and civilians on the ground. The fragmented, commingled, and sometimes burned condition of some bodies would make identifying them extremely difficult.

A team of four forensic anthropologists and two forensic odontologists from the United States military's Central Identification Laboratory (CIL) in Hawaii was deployed to assist with sorting and identifying the dead at Dover Air Force Base in Delaware. But just getting to Dover from Hawaii would prove to be a challenge. The Main Gate at Hickam Air Force Base where the team worked had become a parking lot as security personnel inspected vehicles and pedestrians to ensure there were no threats, either by personnel or objects coming onto the base. The sprawling military base was, in military terminology, at Force Protection Delta, its highest level of security. After arriving on base and selecting the identification team, the next hurdle was getting this team of specialists to Delaware at a time when all aircraft in United States airspace were grounded—there were no available flights from Hawaii to Delaware for four days.

Once cleared for travel, the team flew into Newark, NJ, and spent the next week at the military mortuary in Dover moving from one table to the next, as they employed their unique osteological skills in sorting and identifying body parts and burned fragments of bone, sometimes covered with rubble from the Pentagon. Establishing the personal identity of a human body can pose many difficulties, but the level of difficulty greatly increases if the body or remains are fragmented, commingled, burned, and covered with concrete rubble, jet fuel, and aircraft wreckage. This presentation will cover the role of the CIL forensic anthropologists at Dover, and the information forensic anthropologists are able to glean from examination of human remains, whether fleshed or dry bone, complete and intact, or partial and fragmented.

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### 9/11, Pentagon Attack, Forensic Anthropology

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## A73 A Historical Review of the Recovery and Disaster Victim Identification Protocols Employed in the September 11, 2001, Crash of United Flight 93 in Shanksville, PA, and Lessons Learned

*Dennis C. Dirkmaat, PhD\*, Mercyhurst University, Erie, PA; Marilyn R. London, MA, University of Maryland, College Park, MD*

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**Learning Overview:** After attending this presentation, attendees will have a clear understanding of the roles played by anthropologists in the recovery, analysis, and identification of victims in the September 11 Flight 93 crash in Shanksville, PA.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the use of anthropologists in the response to multiple fatality incidents, both in the field and in the morgue, and especially those with forensic aspects.

On September 11, 2001, a hijacked plane with 44 individuals aboard crashed in southcentral Pennsylvania, instantly killing all aboard. United Flight 93 had left Newark International Airport earlier that morning and was headed to San Francisco International.

Wallace Miller, the coroner in Somerset County, PA, was the first official on the crash scene outside Shanksville, PA. The scene contained fragmented, commingled, and scattered human remains, personal effects, and aircraft wreckage strewn over the mixed forest and open field landscape of approximately 80 acres. One of Miller's earliest decisions was to call a forensic anthropologist, as well as a coroner in the neighboring county, for help. Officials from the Pennsylvania State Police, the National Transportation Safety Board (NTSB), and the Federal Bureau of Investigation (FBI) arrived shortly thereafter.

Given the number of victims and condition of the remains, the best course of action for identification of the victims was to request assistance from the Disaster Mortuary Operational Response Team (DMORT), part of the United States Department of Health and Human Services. DMORT arrived on September 13 and set up a temporary morgue at a National Guard Armory building a few miles away from the crash site.

In terms of recovery and documentation of the victims from the scene, protocols for efficient large-scale outdoor forensic scene recoveries were developed in 2000 that became known as the Weldon Spring Protocols. The original plan at the scene in Somerset County was to put these protocols into action with a combination of local, state, and federal personnel and resources. However, it was decided that since this was a criminal case of national significance, a different course of action for processing the scene should be implemented. After two weeks, recovery operations were completed, and the scene was returned to Coroner Miller. The first visit to the scene, immediately following release of the scene to the coroner's office, however, led to the discovery of more human remains on scene.

A renewed search of the crash site was planned and conducted by volunteers over the course of two consecutive weekends, in the spring of 2002. Significant amounts of human tissue and aircraft wreckage were found. Periodically, throughout the ensuing ten years, additional searches were conducted.

Members of DMORT Region III, from the mid-Atlantic area, were deployed to the scene in Somerset County. DMORT brings its own logistics personnel, pathologists, forensic anthropologists, medical records technicians and transcribers, fingerprint specialists, forensic odontologists, dental assistants, X-ray technicians, and other personnel. DMORT usually provides a fully stocked mobile morgue. However, in 2001 the DMORT system had only one mobile morgue, which was sent to the World Trade Center. Local resources and private companies were utilized to supplement the government's supplies. The DMORT III Team Leader and Morgue Director organized the personnel at each station and wrote up detailed operations protocols in what became the DMORT Morgue Protocols.

The morgue operation, consisting of ten individual stations (both documentation and identification stations), continued smoothly and was completed in 12 days (on the afternoon following the completion of the field operations). More than 1,600 fragments of human remains were analyzed for DNA and matched to 40 crew members and passengers. Four sets of remains were not identified and were turned over to the FBI as presumed remains of the terrorists.

Coroner Miller's lasting contribution to the effort and future efforts was the positive interface with the families of the victims. He utilized his experience as a funeral director to fully embrace their concerns. He met with them individually and as a group, becoming not an adversary but an ally and advocate for family rights.

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### Disaster Scene Recovery Protocols, Disaster Morgue Operations, History of September 11, 2001

## A74 Lessons Learned From the September 11, 2001, Terrorist Attacks and Other Recent Mass Fatality Incidents

*Frank DePaolo, PA-C\*, New York City Office of Chief Medical Examiner, New York, NY; Emily Carroll, MSPH, New York City Office of Chief Medical Examiner, New York, NY; Helen Alesbury, MA, New York City Office of Chief Medical Examiner, New York, NY*

**Learning Overview:** The goal of this presentation is to provide attendees with data and characteristics from recent mass fatality incidents for use in future planning and preparedness efforts. After attending this presentation, attendees will have an appreciation for the complexities of responding to a mass fatality incident and better understand the objectives to successfully manage such an incident.

**Impact Statement:** This presentation will impact the forensic science community by providing data and characteristics on mass fatality incidents that will guide planning and preparedness efforts within their jurisdictions.

This presentation, utilizing data from a study of recent mass fatality incidents, will identify characteristics, trends, and lessons learned that may inform planning and preparedness efforts for the medicolegal community. The study includes several large-scale incidents that, as outliers, have influenced fatality management operations and preparedness efforts on a national level.

It is imperative that medicolegal jurisdictions prepare for the occurrence of a mass fatality incident. The failure to appropriately plan for a mass fatality incident will cause additional delays, potential lost data, jurisdictional confusion, and negatively impact the families and loved ones of victims. Despite the trend to plan for catastrophic and complicated incidents, this analysis of recent mass fatality events seeks to better inform authorities regarding the scale and types of incidents that could potentially impact their jurisdiction. The guidance provided by this study serves as a tool to guide the development of plans, acquisition of appropriate resources, and training of staff.

To perform this analysis, data were collected from mass fatality incidents occurring in the United States from January 1, 2000, to July 31, 2021, that resulted in ten or more fatalities. Specific data points were collected for each incident, including the date, location, number of fatalities, incident type (man-made or natural), incident subtype, and description (mass shooting, hurricane, aviation, etc.). A total of 180 incidents fit the criteria for inclusion in the analysis, resulting in a total of 626,548 fatalities. The average number of incidents was eight per year during the study period. The analysis demonstrates that most mass fatality incidents (86.1%) result in between 10 and 50 fatalities and are variable based on incident type and geographic location. The average number of deaths caused by a single incident was 3,480; however, removing the large-scale outliers (September 11, 2001, attacks, Hurricane Katrina, Hurricane Maria, and COVID-19) lowers this average to 27 deaths per incident. In addition, the data indicates that a majority of incidents involve open populations (83%) with none or partial victim manifests. The top incidents based on the average number of fatalities per incident include Biological (COVID-19), Terrorism (as dictated by the September 11, 2001, attacks), hurricanes, tornados, and aviation incidents. However, the incidents occurring at the highest frequency include tornados, aviation incidents, mass shootings, and hurricanes.

The September 11, 2001, terrorist attacks resulted in 2,753 fatalities associated with the World Trade Center, 184 fatalities associated with the Pentagon, and 40 associated with Flight 93 in Shanksville, PA. The subsequent remains recovery and identification operations, especially associated with the World Trade Center, have served to inform the New York City Office of Chief Medical Examiner (NYC OCME) of the capabilities required to manage a complex, protracted victim identification process involving extensive body fragmentation and commingling. While the World Trade Center Attack, as well as the Pentagon attack and crash of Flight 93, has been shown to be outside the normal trends of mass fatality incidents, it has none the less offered the medicolegal community several invaluable lessons.

Notwithstanding the scale of the incident, the events of September 11 occurred across jurisdictions, resulted in fatalities of numerous nationalities, and required protracted operations lasting for years. The unique nature of these incidents has provided the medicolegal community with valuable knowledge and lessons that can be implemented in future planning and training efforts. The complexities associated with the September 11 terrorist attacks, with special attention to the NYC OCME recovery and identification efforts associated with the World Trade Center, will be compared and contrasted with other more “typical” mass fatality events that have occurred over the past 20 years in the United States.

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### Mass Fatality Incidents, Lessons Learned, 9/11

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## A75 The Role of the Medical Examiner in Two Mass Fatality Events: The World Trade Center Disaster and the COVID-19 Pandemic

*Jason K. Graham, MD\*, New York City Office of Chief Medical Examiner, New York, NY; Barbara Sampson, MD, PhD, New York City Office of Chief Medical Examiner, New York, NY*

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**Learning Overview:** Attendees will be informed about the role of the medical examiner in mass fatality events, using the events of 9/11 and COVID-19 in NYC as examples.

**Impact Statement:** Forensic science has and will continue to learn from the experience of the New York City medical examiner office with respect to mass fatality events of several different kinds in the areas of pathology, anthropology, fatality management, and identification.

The role of the medical examiner is critical in mass fatality events. It includes documenting, recording, investigating, recovering, and processing decedents in a dignified and respectful manner, accurately determining the cause and manner of death, performing the accurate and efficient identification of victims, and supporting the judicial, public health, and investigative objectives and requirements. Depending on the nature of the event, different roles may be predominant. This presentation will offer suggestions for effectively handling a mass fatality event based on lessons learned from previous events.

New York City has experienced the largest homicide rate in United States history, as well as the largest number of fatalities resulting from a natural disease pandemic in United States history. After providing an overview of the Office of Chief Medical Examiner (OCME) of the City of New York, the response to each disaster will be outlined and the experiences compared and contrasted with emphasis on the lessons learned. Details of the World Trade Center (WTC) and COVID-19 responses will include changes to operations in case intake and investigations, case recovery and transport, establishment of disaster portable morgues, and identification of decedents with outreach to their next of kin. The coronavirus response also required significant assistance to overwhelmed hospitals. Discussion of lessons learned will include the importance of technology for improving the response, flexibility with the plan and with deployment of staff, planning for the respectful storage of remains beyond the immediate event, clear messaging and managing expectations with families, media and staff, and early implementation of health and safety measures.

Finally, the importance of wellness and resiliency for staff will be described. Over the past few years, OCME has recognized that the repetitive exposure to traumatic subject matter, compassion fatigue, and cultural pressure have created a unique stress on staff. The agency has prioritized the wellness of agency personnel. The OCME Wellness and Resiliency Program works to develop materials, provide resources, host trainings, and empower staff to build resiliency holistically—focusing not just on mental health, but also considering the emotional, physical, and spiritual health of staff. Staff are encouraged to participate in ongoing activities and trainings throughout the year and take advantage of resources in an effort to build a strong baseline resiliency, both personally and as a work community.

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### Fatality Management, 9/11 Attacks, Medical Examiner



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## A76 The Changing Role of Forensic Anthropology in Investigations of Mass Fatality Incidents

*Douglas H. Ubelaker, PhD\*, Smithsonian Institution, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will be more familiar with the variation of the role of the forensic anthropologist in the investigation of mass fatality incidents

**Impact Statement:** This presentation will impact attendees by making them more aware of the nature of anthropological contributions to the investigation of mass fatality incidents.

Working in their home laboratories and institutions, forensic anthropologists become accustomed to their usual roles and procedures in casework. When anthropologists participate in the investigation of mass fatality incidents, those roles and procedures likely will differ from those they are accustomed to. The magnitude of the mass fatality problem being investigated, and the nature and structure of the team assembled, shape the participation of forensic anthropologists. In such investigations, anthropologists may be assigned specific roles as designated by team leadership, such as assisting in recovery, sorting commingled remains, or assessing age at death. While these roles utilize the unique skills and training of the forensic anthropologist, they may be focused on particular aspects of the investigation. When participating in such investigations, the forensic anthropologist must rapidly become familiar with the defined structure and responsibilities and adapt usual protocols to meet the needs of the investigation. Participation may present significant challenges, especially regarding equipment access, logistics, culture/ language issues, and team dynamics. Although the participation role may be narrower and more focused than the forensic anthropologist is accustomed to, the participant must identify and address any associated ethical issues and apply appropriate methods to the problems assigned. Anthropologists should not perform work that they consider unethical (such as offering opinions beyond their expertise).

This presentation reviews the different contributions made by forensic anthropologists in the investigation of mass fatality events. Perspective from personal experience in contributing to the investigation of the 9/11 Pentagon investigation, as well as other mass fatality events, will be provided. The role of the forensic anthropologist varied considerably in the investigation of fatalities associated with 9/11, the Branch Davidian compound in Waco, TX (1993), Operation Desert Storm (1991), the 1995 bombing of the Alfred P. Murrah Federal Building in Oklahoma City, and the military dictatorship period (1973-1990) in Chile. All of these investigations involved a team approach, but each presented unique challenges and professional demands for the forensic anthropologist. Although the institutions and teams involved were highly variable, specialists worked well together. Involvement in such complex endeavors offers an opportunity to contribute to issues of great importance. Such involvement also provides an educational experience to all involved.

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### Forensic Anthropology, Mass Fatality, 9/11 Pentagon

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## A77 20 Years of DNA Developments for World Trade Center Victim Identification

*Mark Desire, MS, JD, New York City Office of Chief Medical Examiner, New York, NY; Carl Gajewski, MS, New York City Office of Chief Medical Examiner, New York, NY; Andrew Schweighardt, PhD\*, New York City Office of Chief Medical Examiner, New York, NY*

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**Learning Overview:** After attending this presentation, attendees will be more familiar with developing DNA techniques to make identifications from a mass fatality.

**Impact Statement:** This presentation will demonstrate the commitment to not giving up and how we can learn from failure to reach our goals.

The New York City Office of Chief Medical Examiner (OCME) continues to fulfill their promise to the families of the victims of the 2001 World Trade Center attack—a promise to use every means necessary to identify their loved ones. From day one, the families looked to the OCME for hope. They continue to put their trust in the OCME today and this has not been forgotten. Through the challenges of working on the country's largest forensic investigation on some of the most degraded of human remains, this study set out to explore the 20 years of pushing forward and evolving DNA methods from lessons learned.

Two thousand, seven hundred fifty-three individuals were killed that morning, shattering the victims into 21,905 pieces scattered over many square blocks. The OCME had the immense job of identifying these victims.

DNA testing on each bone fragment has been attempted multiple times over the past 20 years. From the first step of pulverizing the bone into a fine powder, to decalcifying the cells and extracting what little DNA is left, each step has been modified and advanced.

The first phase yielded a 38% success rate, meaning that DNA profiles were generated with enough alleles to reach the statistical threshold to identify. Not all remains were linked to a reference sample, but at the very least, they could be identified should one be received in the future. The second phase of DNA testing saw a change in the Short Tandem Repeat (STR) kit and a more robust bone grinding process. As a result, the success rate increased to 45%. The third phase began with the use of mini-STRs and a new bone optimization DNA extraction protocol. The success rate increased to 54%. These samples were the most degraded and had been attempted during the previous two phases, which means the increased success rate was achieved on samples that previously yielded no results. The fourth phase began in 2006 with 6,317 bones remaining negative from prior attempts. With new grinding protocol, demineralization, and amplification technique, a 59% success rate has been observed. In addition, Y-chromosomal Short Tandem Repeat (Y-STR), mitochondrial DNA, and Single Nucleotide Polymorphism (SNP) testing have all played a role during the four phases. As advancements in testing protocols have been made, the results continue to improve, even when only the most challenging samples are left.

Some 2,400 fragments still provide little to no information. These are the remains that have been the most severely degraded by fire, water, heat, mold, bacteria, insects, jet fuel, diesel fuel, and sunlight; all factors present at Ground Zero, making this not only the largest investigation, but also the most difficult.

The necessity to improve protocols led to something else—using the experience gained to aid in the daily body identification cases, and to share this knowledge with laboratories all over the world. DNA testing is the most conclusive mode of identification of fragmented and degraded human remains. Through the efforts of the OCME to identify the unidentified, while equipped with experience garnered from 20 years of effort, this staff of scientists is well prepared to optimize and disseminate this knowledge to relevant communities.

Further expansion on these techniques is possible, giving the ability to identify victims of mass fatalities that are impossible using traditional methods. New technologies in the DNA field are rapidly evolving. Next-generation DNA sequencing is one such technology that can be applied to accelerate victim identification by enabling the comprehensive analysis of DNA to become inexpensive and routine. These capabilities are the next step for World Trade Center identification 20 years later.

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### DNA, 9/11, Bone

## A78 World Trade Center Data Analysis Project: 20 Years of Ongoing Identification Efforts

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**Learning Overview:** For the past 20 years the New York City Office of Chief Medical Examiner (OCME) has been continuously working to identify the World Trade Center (WTC) victims who died on September 11, 2001. The work continues to this day. This presentation provides the results of a data analysis project focusing on the identification trends from 9/11/2001 to 9/11/2021. The presented data were analyzed in collaboration with scientists at the Defense POW/MIA Accounting Agency.

**Impact Statement:** For 20 years, the OCME has been analyzing victims' remains from the 9/11 WTC attack in order to identify as many individuals as possible. This presentation presents the findings from an analysis of these data spanning 20 years, specifically pertaining to victim identification status and body fragmentation, among other parameters. The results will be useful for managing expectations with other large-scale mass fatalities.

On September 11, 2001, two planes struck the WTC towers, leading to their eventual collapse. The terrorist attack resulted in the death of 2,753 known victims and the eventual recovery of 21,905 remains. Based on data pertaining to last known location, there are 1,510 victims associated with Tower 1; 736 victims associated with Tower 2; 87 victims associated with American Airlines Flight 11 (AA11) that impacted Tower 1; and 60 victims associated with United Airlines Flight 175 (UA175) that impacted Tower 2. An additional 360 people died at different locations surrounding the WTC site, in hospitals, or their location at the site is unknown. The reported values only reflect victims of 9/11 and do not include terrorists.

As of September 2021, the OCME's efforts have resulted in the identification of 14,750 remains. Currently, there are still 1,106 known victims (40%) who have never had any remains identified to them. Results show that 42% of the victims associated with Tower 1, 40% from Tower 2, 24% from AA11, and 53% from UA175 remain unidentified.

Results show there is an association between last known location in the towers and identification status. Fewer individuals have been identified from the floors impacted by the airplanes, specifically Floor 97 in Tower 1, which has a 34% identification rate, and Floors 82 and 83 in Tower 2, which have 23% and 30% identification rates, respectively. The largest number of victims from a single employer worked at Cantor Fitzgerald, located in Tower 1 on Floors 101–105, just above the impact site. To date, 285 of the 658 victims from Cantor Fitzgerald (43%) have never been identified. Aon, located in Tower 2 just above the impact site on Floors 92, 93, and 98–105, has 51% of employees unidentified (88 unidentified; 87 identified). While the exact location of all individuals cannot be known at the time the planes struck or the towers collapsed, data indicate a lower identification rate associated with the areas of impact and above.

Among the WTC victims, there is a wide range of body fragmentation and variability in body completeness. While most identified victims have had less than 10 remains associated to them, some individuals have had over 100 linked fragments. Data indicate that bodies associated with lower floors are more complete than from upper floors. The high degree of body fragmentation has led to a heavy reliance on DNA testing. Approximately 93% of the identified remains (13,725) have involved DNA analysis. As DNA testing becomes more sensitive, remains are periodically retested if they did not previously produce a viable profile. Currently, there are 3,404 remains undergoing additional DNA testing. Only unidentified remains with associated bone were selected for ongoing testing.

DNA testing provides three possible outcomes: (1) a new identification (first time remains identified to a victim); (2) linked remains (additional remains linked to a previously identified victim); or (3) insufficient DNA (profile not generated). Trends show that the number of new identifications was highest from 2001 to 2003 and by 2005 had plateaued, with most DNA results linking to previously identified victims. Between January 2006 and September 2021, there have been 3,798 links to previously identified individuals and 49 new identifications. Using an Identification Accumulation Curve (*c.f.* species accumulation curve) and the Good-Toulmin Estimator, an additional 76 +/- 55 (95% confidence interval) new identifications are estimated from the 3,404 samples, and the remainder would be links to previously identified victims. These results will be useful for managing expectations as the identification work continues.

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### 9/11, Victim Identification, World Trade Center

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## A79 Victim Identification and the Notification Process: A 9/11 Family Member Perspective

*Mary Fetchet, LCSW\*, Voices Center for Resilience, New Canaan, CT*

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**Learning Overview:** Through this presentation, attendees will learn the complicated issues that victims' families experience following acts of mass tragedies and recommendations for improving the process and helping them navigate.

**Impact Statement:** Victims' families require access to detailed information, support, and guidance during their initial and ongoing processes related to the identification and notification process of their loved ones' remains. They often require emotional and bereavement services subsequent to the notification from an organization outside of the medical examiner's office and other authorities involved with other aspects of the crime.

On September 11, 2001, 2,977 innocent individuals were murdered when 19 terrorists used four passenger planes as weapons to attack the United States. Two thousand, seven hundred fifty-three died at the World Trade Center (WTC) site, 184 at the Pentagon, and 40 on United Airlines Flight 93 in Shanksville, PA. The victims' families were geographically dispersed around the United States and in 90 countries abroad. This presentation will focus on the victims' family members whose loved ones died at the WTC site.

In the days following the attacks, family members were uncertain if their loved one was dead or alive. Accessing information was challenging, and providing DNA samples was complicated. The recovery effort at the WTC site continued for nine months at multiple locations, lengthening the collection of body parts, which impacted the identification and notification process. Despite these challenges, the New York City Office of Chief Medical Examiner (OCME), led by Dr. Charles Hirsch, made a promise to the families that they would continue the identification and notification process indefinitely.

In the immediate aftermath, a group of 9/11 family members met regularly with the OCME for updates on the collection of human remains and advocated for a streamlined notification process. This process empowered family members to determine if, when, and how they received notifications. Some families chose to be notified only once and some families chose to be notified every time there was a new identification. Some families decided to have their loved ones remains in the repository located within the 9/11 Memorial.

Beyond the complexities of the notification process, victims' families had to navigate complex systems and issues at a time when they were grieving. Funerals, religious rituals, and burials were delayed. Memorial services were held without bodies to bury. Because families were geographically dispersed, local authorities often conducted the collection of DNA samples and made notifications about new identifications. Often these local officials were untrained and support services were not provided to help guide the family. Multiple notifications have led to complicated decisions related to the burial process. In some circumstances, severed relationships between family members, especially spouses and parents of the deceased, have led to questions about the transfer of rights from the next-of-kin to claim remains.

Twenty years following the attacks, over 1,100 of the 2,753 victims at the WTC site have not yet been identified. Dr. Barbara Sampson, Dr. Hirsch's predecessor, and her dedicated team at the OCME have maintained the promise that the identification process will continue. With the passage of time, special consideration has to be given when making new notifications to the next generation of families. In retrospect, the OCME staff has demonstrated caring and compassion in their interactions with 9/11 families. Through the OCME's collaboration with Voices Center for Resilience (VOICES), the families have been supported throughout the notification process to help mitigate complicated mental health issues that many families experience due to the horrific deaths of their loved ones.

In the aftermath of 9/11 and other acts of mass violence, victims' families are grieving, yet they are often required to navigate complicated issues related to the processes in identifying, recovering, and interring the remains of their loved ones. This presentation will cover the first-hand experience of a 9/11 family member, and the perspectives she's gained through her work in providing long-term support for thousands of 9/11 families. This presentation will also draw upon her expertise in supporting communities impacted by other tragedies and parallels to the experience of those who recently lost a loved one following the COVID-19 pandemic.

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### 9/11 Victims' Families, Human Remains Notification, Bereavement

## A80 Geophysical Imaging of Buried Human Remains in a Mass Grave Experimental Setup

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**Learning Overview:** After attending this presentation, attendees will have learned how measured geophysical anomalies associated with a mass grave experiment vary with time after burial. A comparison of temporal changes in geophysical signals with soil and climatic variables will also be presented. Ultimately, this presentation will highlight and compare the use of different non-invasive geophysical techniques, including electrical resistivity tomography, electromagnetic, self-potential and ground penetrating radar for locating mass graves and monitoring the spatio-temporal changes around them.

**Impact Statement:** Robust and reliable non-destructive (i.e., without excavating) methods of locating mass graves are important as logistical restrictions and security issues can mean that destructive techniques are not always feasible or desirable options. This project will contribute to existing knowledge on the detection of mass graves by monitoring landscape/soil changes throughout the experiments using geophysical techniques and by evaluating the validity and reliability of these methods to detect the presence of a mass grave with human bodies throughout different stages of decomposition.

Geophysical methods provide non-destructive approaches for detecting anomalies that provide investigative leads to clandestine individual and mass graves.<sup>1,2</sup> Studies on investigating the use of geophysical techniques for locating graves and monitoring human decay have mostly relied on proxies such as pigs, while case applications, though with promising results, are not without uncertainties.<sup>3,4</sup> The success rates have been dependent on seasonal variations, with their effects yet to be rigorously tested. These create uncertainties with open questions such as: How do geophysical anomalies resulting from buried human remains vary with time as the body decays? How do changes in soil properties around decaying bodies correlate with measured geophysical signals? What effects do climate variables have on measured geophysical signatures? What methods are more suitable at the different decay stages? To investigate these research questions, this study hypothesized that the burial and decay of human remains with time cause changes in the soil physical and biochemical properties, including electrical conductivity, temperature, pH, and redox potential around them. These changes in contrast to their surrounding soils create measurable anomalies in the soil bulk geophysical responses, including electrical conductivity and dielectric permittivity.

To investigate the above hypothesis, this study was conducted as part of a novel, interdisciplinary mass grave experiment established in May 2021 consisting of a mass grave with six willed/donated human remains, an empty control mass grave, three individual graves with a human remain in each, and an empty control individual grave. Prior to establishing the graves, this study conducted background measurements of Electrical Resistivity Tomography (ERT), Electromagnetic (EM) and Ground-Penetrating Radar (GPR) while soil profiles were analyzed *in situ* after excavating the graves. All the graves are also instrumented with soil sensors for monitoring temporal changes in soil moisture, temperature, and electrical conductivity *in situ*. Geophysical measurements of ERT, EM, and GPR were repeated two days, as well as one and two months, after burial while further measurements are planned on a bi-monthly basis for the next 15 months.

Results obtained to date show over a 20 percent decrease in electrical conductivity measured from EM imaging and an increase in resistivity measured from electrical resistivity imaging immediately after burial with the resistivity decreasing over time within the graves with human remains. GPR profiles also consistently show distorted reflectors and hyperbolas indicative of ground disturbances and the presence of human remains.

This presentation will highlight the experimental design, field implementation, and results of this study and discuss their implications for using geophysical methods as non-destructive techniques to guide the detection of clandestine graves.

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### Forensic, Geophysics, Mass Grave

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## **A81 An Actualistic Experimental Replication of a Small-Sized Mass Grave: A Taphonomic Study Aimed at Developing Virtual Training Tools, Advancing Methods of Human Identification, and the Detection and Documentation of Mass Graves**

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**Learning Overview:** After attending this presentation, attendees will have learned how actualistic experiments can contribute to the refining and testing of methods and techniques used during the investigation of mass graves, as well as training in mass grave excavation and documentation. This presentation highlights how expertise from forensic archaeology, forensic anthropology, forensic isotopes, forensic chemistry, forensic proteomics, metabarcoding, geophysics, human remains detection canine research, and remote sensing are combined to support humanitarian forensic science.

**Impact Statement:** The presentation aims to contribute to the development, testing, and validation of scientific methods for mass grave investigation by increasing current knowledge and understanding of mass grave taphonomy. Specific objectives include developing and refining detection and documentation techniques, investigating the potential of proteomic and microbial biomarkers for more precise and accurate Postmortem Interval (PMI) and age-at-death estimation, and evaluating diagenesis and alteration of isotopic signatures of different human tissues. Each of the subprojects will generate recommendations for documentation, sampling, and analysis protocols. These, as well as the 3D documentation of the donors and graves during the experiment, form the basis for a 3D virtual training tool that will function as a teaching aid to demonstrate the complex taphonomy of mass graves and provide guidance on choices made during excavation.

This presentation introduces an actualistic study replicating a small-sized mass grave with donated human remains at the Forensic Anthropology Center, Texas State University (FACTS) and presents the results of the first phase of development of a 3D virtual training tool for mass grave excavation and documentation. The “Mass Grave Project” aims to advance methods of detection, documentation, and analysis of mass graves and the human remains within them. The experiment is designed to enable comparison of mass grave and individual grave contexts and increase understanding of the effects of the environment and taphonomic variables, such as weather and soil properties. It examines the effects of burial and decomposition of multiple bodies in close proximity on the preservation and recovery of human tissue biomarkers and potential transference between individuals within mass graves. This project includes four core research areas (subprojects): (1) detection and location, (2) documentation, (3) methods supporting human identification, and (4) training—development of a virtual 3D training tool.

In May 2021, a mass grave (six donors), a control mass grave (no donors), three individual graves, and one individual control grave were placed at the Forensic Anthropology Research Facility, San Marcos, TX. The graves are being monitored *in situ* over a period of 18 months, after which they will be excavated. Human tissue and environmental (soil, vegetation) samples were collected prior to burial. Soil samples, Volatile Organic Compounds (VOCs), and remote and geophysical data are being collected from the graves at intervals throughout the experiment. Temperature, moisture, and electrical conductivity are monitored by soil sensors. Prior to and during burial, the donors, graves, and environment were documented in 3D using Computed Tomography (CT) scanning and photogrammetry. The results of the first three subprojects, combined with the 3D data generated in the experiment, provide research-based educational materials that are used to develop a 3D virtual training tool for documentation and excavation of mass graves.

The 3D virtual training tool will be made available to international humanitarian organizations involved in mass grave investigation. The first phase of development of the training tool consists of a 3D virtual environment combining data from different 3D documentation methods (CT scanning, [aerial] photogrammetry, 360-degree environment capture, laser scanning) collected prior to and during burial. The 3D environment and interactive modules are developed using 3D modeling, animation, and videogame engine software. This presentation demonstrates the virtual environment and shows how initial results from the subprojects are incorporated into the training tool.

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### **Mass Grave, Experimental Taphonomy, Method Development and Training**

## A82 Open Source Geographic Information Systems (GIS) Models for Clandestine Mass Grave Prediction in Developing vs. Industrialized Countries

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how open source tools can assist in the location of mass graves in both developing and more industrialized countries.

**Impact Statement:** This presentation will impact the forensic science community by comparing the application of GIS to the location of clandestine graves in two countries, developing and industrialized, that have undergone conflict.

The location of clandestine graves, whether individual or mass, is often critical to prosecution of perpetrators, community recovery, and familial closure. Traditional methods of post-conflict grave location include, among others, witness testimony, extensive ground searches with probes and/or ground penetrating radar, and expensive purchases of satellite imagery, if available. While the use of GIS for grave mapping has been attempted in the post-conflict context, these previous efforts have primarily relied on commercially purchased and licensed GIS programs, which may be beyond the economic means of most Non-Governmental Organizations (NGOs) and families searching for missing persons.<sup>1</sup>

Using open source data, systems, and tools, such as the open source QGIS and open source maps, proved to be user friendly for both countries used in this comparison.<sup>2</sup> Organizations or groups wanting to explore the use of these tools may not feel intimidated by the complexity or programs requiring purchase and licensing. Additionally, using QGIS allowed manipulation and visualization of data to gain further insights.

In Guatemala the civil conflict and killings originated in the 1960s and lasted until the 1980s, revolving around both ethnic and socioeconomic factors; conflict predominantly occurred in less-developed rural areas. In this country, the intersection of roads, waterways, and points of interest (e.g., churches, plazas, etc. within possible towns, found by use of transformations in QGIS) were the best predictors of clandestine grave locations.<sup>3</sup> These three features eliminated ca. 90% of the geographic area of the country. Thus, this model alone decreased the area needing to be searched and defined its boundaries, providing both searchers on the ground and those using satellite imagery considerably less territory to cover.

In Bosnia, as expected, the model generated from the Guatemalan data did not perform well due to several factors, including the greater infrastructure in the areas of the country where killings (1991–1995) took place. The intersection of roads, waterways, and points of interest in Bosnia eliminated almost none of the country's area. Another difference affecting the model was the number of victims per grave, which ranged from 40 to 1,751, whereas in Guatemala, the model included graves containing 5 to 150 victims.<sup>4</sup> The Bosnian data also links victims found in clandestine graves to known execution sites, primary graves, and secondary graves, enabling the exploration of distances from origin to disposal. These differences highlight that background information (e.g., resources available to the perpetrators) and the geographic nature of the conflict is crucial to model development and more studies of this type need to be conducted on existing data in order to refine and enhance the utility of predictive models.

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### Open Source, GIS, Mass Grave

## A83 The Detection of Clandestine Graves in an Arid Environment Using Unmanned Aerial Vehicle (UAV) Remote Sensing

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the effectiveness of using different imaging techniques assisted by UAVs in narrowing the search area of possible recent and old gravesites in arid environments.

**Impact Statement:** This presentation will impact the forensic science community by improving the current grave detection methods in arid environments, which will be an excellent addition for all the agencies involved in the detection of burial sites.

Several countries in the Middle East have suffered from armed conflicts that resulted in many clandestine mass graves.<sup>1</sup> Covering a large land area and thoroughly searching it for a possible burial site places a significant demand on both human and financial resources.

This research was carried out in Kuwait, a country known for its arid environment, using different imaging sensors mounted on UAVs to help improve the current grave detection methods in an arid climate. Multiple single graves and a mass grave with sheep carcasses were simulated in a fenced Kuwaiti desert area. Pigs have always been the first analog choice to simulate human bodies when it comes to conducting forensic experiment; however, since pigs are not available in Kuwait, sheep would be the closest equivalent available to use.<sup>2</sup> It is not the first time other mammals have been used when conducting such experiments.<sup>3</sup> The results obtained from the analysis of the acquired data confirm the effectiveness of such technology in detecting burial sites. They support and favor the proposed aims and objectives of the research. Differences in elevation between the graves and their surroundings were identified. Also, the analysis of the images captured with the multispectral sensor revealed a slightly noticeable variation in the Normalized Difference Vegetation (NDVI) index. Furthermore, both the Red/Green/Blue (RGB) and multispectral sensors were used successfully to detect surface anomalies on and around the graves.

The results from analyzing the data captured using thermal imaging also support the view that temperature variation between the graves and their surroundings exists. Subsequently, the calculation of Temperature Vegetation Dryness Index (TVDI) confirmed the effect of buried cadavers that produced a difference in soil moisture in the grave area.

The goal of this research is to develop a cost- and time-effective burial detection method that can be used in an arid climate.

This research affects many countries in the Middle East region, which in the past decades have suffered from war conflicts and human rights violations resulting in many clandestine graves. It is hoped that the present research will help locate these graves and identify the missing individuals, bringing closure to their families.

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### Forensic Anthropology, Remote Sensing, Clandestine Graves



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## A84 Continuing to Shift the Forensic Anthropology Paradigm: Let's Be Realistic!

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the recent paradigm shift in goals and roles of forensic anthropology within the medicolegal community.

**Impact Statement:** This presentation will impact the forensic science community by presenting a detailed review of the state of the field of forensic anthropology in 2022.

A few years ago, American Forensic Anthropology stepped back to reconsider its components as a scientific discipline. One of the most visible results of this reevaluation was the change of the name of the corresponding AAFS section, from Physical Anthropology to just Anthropology. Most immediately, this change was intended to acknowledge the growing importance of forensic archaeology and forensic taphonomy as two of the essential components of the field. The adoption of these two components in the past three decades increased the range and depth of data collection in the field and the hypotheses that this discipline was able to construct and confront. At a deeper level, the change implicitly acknowledged a paradigm shift in forensic anthropology, from an applied, lab-based discipline aimed exclusively at victim identification to a much broader fully scientific one concerned also with the reconstruction of past events.

These changes have generated healthy and fruitful discussions on the most academic aspects of this field, from desired research lines or course curricula to changes in the professional certification requirements. However, it is also necessary to again take a step back to consider how these changes should translate into the practical role in the investigative process and within the forensic community. This is especially true with respect to the needs and expectations of those stakeholders whom we are supposed to assist in that process. Why do law enforcement and coroners invite us into the forensic investigation? What do they think forensic anthropologists can add to the investigative process, and what in our specific skillset can meet those needs and expand those expectations to better fit what modern forensic anthropology can actually offer?

The answer to these questions reveals that it may be useful to divide the skillset of forensic anthropology into two different but interrelated sets of capabilities, useful both to guide the development of this field and to communicate more efficiently what forensic anthropologists can do for coroners, medical examiner offices, and law enforcement agencies.

The first subdiscipline or set of capabilities is the classic one, focused on aiding in victim identification through osteological analyses. It is likely what comes to the mind of law enforcement and other medicolegal professionals when thinking of forensic anthropology. Constructing a biological profile, noting unique features to help narrow the missing person list; and maybe analyzing bone trauma at the lab. This subdiscipline can be called Biological Profile Assessment.

The other set of skills and subdiscipline is concerned with reconstructing the history of, primarily, the outdoor scene. This is termed Outdoor Crime Scene Reconstruction, which refers to the reconstruction of the chain of events surrounding death and emplacement of the decedent, focusing primarily on the role that humans may have played in those events and during the forensic taphonomic interval. This component requires the classic osteology skills for evaluating forensic significance, and subsequent laboratory analysis of biological profile and trauma analysis. But it also requires forensic archaeology for locating and documenting spatial distribution of the evidence, scene and event context, and hypotheses of association. Finally, it requires forensic taphonomy to tie the room together and provide the background, data, and scope (from disciplines apart from forensic anthropology) to scientifically assess the potential human role in the death, deposition, and post-depositional disturbance events.

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### Forensic Anthropology, Forensic Taphonomy, Outdoor Crime Scene Reconstruction

## A85 Pair-Matching Commingled Femora: Applying Geometric Morphometrics to Detect Shape Similarities in Human Femora

Christina Recheis\*, Cranfield Forensic Institute, Cranfield University, Shrivenham, Oxfordshire, United Kingdom; Roland Wessling, MSc, Cranfield Forensic Institute, Cranfield University, Shrivenham, Oxfordshire, United Kingdom

**Learning Overview:** After attending this presentation, attendees will gain an understanding of how geometric morphometrics can be applied to digitally collected landmark datasets and how it can be utilized to pair-match human femora and aid in the resolution of commingled cases.

**Impact Statement:** This presentation will impact the forensic science community by presenting the new approach of resolving commingled remains based on shape similarities of paired elements by using 3D models from scan data. It provides a basis for increasing the accuracy of pair-matching and extending it to other skeletal elements. By using 3D models generated from Computed Tomography (CT) scans or scans from structured light scanner systems, a simultaneous digital collection can be created that can be accessed anywhere and shared more easily among investigators.

Commingled remains are found in a multitude of contexts, such as mass graves, mass disasters or ossuaries. Large-scale commingled cases especially can pose major challenges and their resolution is dependent on the examining experts and available resources. With the progressing digitization of science, virtual anthropology offers an application possibility for analytical methods like geometric morphometrics that can help face challenges like the resolution of commingled remains. Applying shape analysis to the investigation of bilateral (a)symmetry has already been shown to be effective in certain commingled cases for some skeletal elements when used with 2D images and manual landmark selection.<sup>1</sup>

Geometric morphometrics is a landmark-based approach to analyze the shape of a structure. Based on 2D or 3D landmark data in the form of Cartesian coordinates, shape is preserved and compared between two samples to evaluate their distance, called the Procrustes distance to each other.<sup>2</sup>

The aim of this research is to show that geometric morphometrics can be used to identify the femora of one individual among commingled remains. It aims to show how landmark data can be collected digitally and semi-automatically from 3D femur models as input for the geometric morphometrics method. Semi-automatic detection of landmarks using an algorithm limits the inter- and intra-observer error as opposed to a completely manual selection. This presentation demonstrates how this can be used as a criterion for pair-matching femora and reducing the inter-observer error inherent with approaches like visual pair-matching.

This study analyzing 18 landmarks and 8 shapes across the femoral surface show a statistically significant ( $p < 0.001$ , single-factor Analysis of Variance [ANOVA]) shape difference between correctly matched and incorrectly matched pairs. This already reduces the number of potential matches down to a few possible pairs that the anthropologist must investigate and rule out based on other approaches.

Thus, geometric morphometric offers a valuable addition to conventional methods and is a promising tool in the investigation of commingled remains.

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1. Garrido-Varas, C.; Rathnasinghe, R.; Thompson, T.; Savriama, Y. (2015) A new method to Pair-match Metacarpals Using Bilateral Asymmetry and Shape Analysis. *Journal of Forensic Sciences*, 60(1).
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### Geometric Morphometrics, Pair-Matching, Semi-Automatic Landmark Detection

## A86 Digital Pair-Matching Based on a Mesh-to-Mesh Comparison of 3D Images

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**Learning Overview:** After attending this presentation, attendees will have learned how to perform a pair-matching of bilateral bones via mesh-to-mesh comparisons using 3D image data. Additionally, attendees will understand that this new method will be useful for quantifying the degree of asymmetry of paired bones as well as segregating commingled skeletal remains recovered in a forensic context.

**Impact Statement:** This presentation will impact the forensic science community by suggesting a new objective pair-matching method that will not only enhance the accuracy of segregation of commingled remains but also overcome the drawbacks associated with traditional pair-matching methods.

Forensic anthropologists often encounter a challenge where multiple human skeletal remains are recovered in a commingled state. Without accurate segregation of commingled remains into individuals, the accuracy of subsequent forensic analyses will be compromised. Pair-matching is one of the popular segregation methods based on the similarity/dissimilarity of the shape and size of bilateral bones. The idea behind the method is that the within-individual discrepancies of the bones should be smaller than the between-individual discrepancies. Traditional pair-matching methods have their own limitations. For example, visual pair-matching is not free from an issue of subjectivity and its accuracy likely depends on the researchers' personal training and experience level significantly.<sup>1</sup> Osteometric pair-matching also has multiple statistics-related drawbacks.<sup>2</sup> The purpose of this research is to generate a new objective pair-matching method based on the mesh-to-mesh comparisons of bilateral bones using their 3D images

Computed Tomography (CT) images of the upper and lower limb bones from 106 Korean individuals (55 females, 51 males) were obtained from the Digital Korean Project (<http://dk.kisti.re.kr/>). The mean ages are 50.9 years (SD=9.8 years) and 53.9 years (SD=8.1 years) for males and females, respectively. After mirroring all left bones using Meshmixer®, each of the mirrored bones were overlapped/aligned with the corresponding right bones from the same individuals using CloudCompare®. In addition, 94-150 pairs from different individuals were randomly selected and compared in the same way. Then, the mean and standard deviation of the distances ( $D_{\text{mean}}$  and  $D_{\text{sd}}$ , respectively) between corresponding faces of the overlapped bones were calculated. To calculate the probability of a pair of bones belonging to a single individual or different individuals, the logistic regression analysis was carried out where the  $D_{\text{mean}}$  and/or  $D_{\text{sd}}$  were used as variables.

The results show that both the  $D_{\text{mean}}$  and  $D_{\text{sd}}$  are lower in the within-individual pairs (0.55mm-0.85mm and 0.48mm-0.78mm for  $D_{\text{mean}}$  and  $D_{\text{sd}}$ , respectively) than in the between-individual pairs (1.14mm-3.12mm and 0.89mm-2.09mm for  $D_{\text{mean}}$  and  $D_{\text{sd}}$ , respectively) for all bones. Overall, the  $D_{\text{mean}}$  and  $D_{\text{sd}}$  are lower in females than males, which indicates that female bones tend to be more symmetric. Within an individual, the lower limbs tend to be more asymmetric than the upper limbs. Logistic regression models yield the Correct Classification Ratios (CCR) ranging between 89.8% (variable:  $D_{\text{sd}}$  of radius) and 100% (variable: both  $D_{\text{mean}}$  and  $D_{\text{sd}}$  of humerus) for males and between 74.6% (variable:  $D_{\text{sd}}$  of humerus) and 96.0% (variable:  $D_{\text{mean}}$  and  $D_{\text{sd}}$  of tibia) for females.

This study suggests a new method to quantify the asymmetry/symmetry of bilateral bones using 3D images of the Korean population. The result of this study will particularly contribute to an accurate segregation of commingled skeletal remains in an objective way.

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### Digital Pair-Matching, Commingled Skeletal Remains, Korean

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## A87 Expanding Lines of Evidence: Contextualizing Open Forensic Cases to Enhance Identification Efforts in Texas

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**Learning Overview:** After attending this presentation, attendees will better understand of the importance of pursuing contextual identifications for long-term unidentified decedents.

**Impact Statement:** This presentation will impact the forensic science community by exemplifying how unresolved forensic anthropological cases in Texas can be further characterized using geospatial, archival, and biocultural data to further identification efforts.

Marginalization and structural inequities that affect people in life can also impact their postmortem treatment in the investigative sphere. Disparities in resource allocation, case prioritization, and conscious and unconscious biases within forensic science, law enforcement, and medicolegal agencies influence the degree of sustained focus a case may receive. Particularly for the long-term dead, a lack of traceable antemortem information, absence of comparative DNA samples, and limited postmortem data often make positive identification extremely difficult, if not impossible. The current presentation argues, however, that investigating these cases should remain a priority and should be carried out by incorporating additional lines of evidence that go beyond the traditional biological profile.

This presentation explores the potential use of non-traditional contextual information via open forensic cases currently housed at the Forensic Anthropology Center at Texas State University (FACTS). The study examines a selection of open cases, ranging from 1967 to 2014, representing at least 81 unidentified individuals who have been transferred to FACTS custody from various medicolegal agencies and legal jurisdictions. cursory analyses suggest that 18 of these cases (22.2%) are likely archaeological in origin and should be repatriated accordingly, while one case is non-human and has been de-accessioned. The remaining 62 cases are of medicolegal significance, and, of these, only 25 (40.3%) have recorded dates of recovery, while 45 (72.6%) have provenience data in the form of county jurisdictions. Fifty-two cases (83.9%) also have associated case numbers from law enforcement or other agencies, while six cases (9.7%) have associated law enforcement reports, and four (6.5%) have available scene photographs. Eight cases (12.9%) have evidence of DNA submission, but only two cases (3.2%) have traceable National Missing and Unidentified Persons System (NamUs) case numbers. These inconsistencies in case documentation highlight systemic roadblocks to identification that often characterize the long-term unidentified, especially in academic institutions where personnel turnover is steady and there is an overreliance on institutional memory.

This research seeks to utilize geospatial, demographic, and records data in order to generate a more holistic characterization of this unidentified population to inform potential new investigative directions. By cross-referencing state and county missing and unidentified persons data in Texas with geographic information on socioeconomic status, mental health, Uniform Crime Reporting statistics, and migration and migrant death rates, the present study examines various biocultural profiles that could potentially supplement unknown persons cases with additional pathways to identification.

The goal of this presentation is to demonstrate the potential application of contextual data to unresolved casework. The integration of disparate types of biocultural, geospatial, and archival information may serve to further characterize unknown individuals when no positive identification is forthcoming, even after the biological profile has been estimated, the remains have been entered into NamUs, and DNA samples submitted. The prioritization of long-term open cases is a way to pursue expanded humanitarian definitions of identity and justice, while broadening the role of forensic anthropologists as scientific investigators and identification specialists.

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### Forensic Anthropology, Contextual Identification, Open Case Investigation

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## A88 Addressing Informational Gaps in Family Reference Sample Practices for Transnational Missing Persons' Cases

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**Learning Overview:** After attending this presentation, attendees will understand how a novel social science research strategy was used to document challenges, leading to the development of informational resources.

**Impact Statement:** This presentation will exhibit an educational resource to address informational gaps in family reference sample collection practices for transnational DNA identification.

When people go missing in United States border states, families and professional stakeholders may not know how best to report a missing person or how to provide family reference samples for DNA identification of deceased migrants. Navigating a lengthy, open-ended missing person search, both families and stakeholders would benefit from improved understanding of family reference sample management practices and increased awareness of the options for families seeking their relatives.

To better understand the challenges faced in cross-border DNA identifications, the study team developed an empirical research strategy to collect qualitative and quantitative data to assess stakeholders' challenges, varying priorities, and points of agreement on solutions, as well as gaps in data necessary to inform policies and practices. In 2020, 26 professional stakeholders were convened for a semi-structured "stakeholder forum." This strategy combined a focus group approach to gather perspectives from stakeholders with similar interests with a community forum approach to collect themes. A pre-forum questionnaire, the forum discussions, and audience response questions were collected and coded for challenges and proposed solutions, yielding 79 challenges and 36 solutions. The challenges and solutions were formulated into 44 challenge statements under six categories and 24 solution statements under four categories for post-forum testing in a modified-Delphi analysis. Stakeholders attending the forums were invited to rank the priority of challenge statements and viability of solution statements via Likert scales, providing comments on their reasoning. Rankings from the 15 respondents were evaluated for consensus across stakeholder types, and comments were assessed for commonalities and differences. Ten of the challenges were grouped for ranking under "Education and Communication," the top two with consensus being "long turn-around time for matches" and "inadequate communication with families while investigations are ongoing."

Partly motivated by the need for improved communication on these practices, the academic BorderDNA working group formed to develop unbiased educational resources on three related applications of DNA at the United States border, including for transnational missing persons. Guided by a master menu of target audiences, product types, and dissemination outlets, the group created an animated video short for families on how to provide family reference samples for transnational DNA identifications. The video aims to educate viewers on: (1) the overall process for use of family reference samples to identify deceased persons; (2) the options available to families to provide family reference samples to the Combined DNA Index System (CODIS) or a private database; (3) the privacy and security protections afforded DNA data; and (4) who should provide family reference samples.

An effective resource should not only address educational gaps; it should also be calibrated to reach audiences enmeshed in a multi-stakeholder context. This entails an unbiased presentation of facts and contexts that remains compassionate and trauma informed. The working group innovated an iterative process to develop and test the content of its resources. First, drafts are shared within the neutral academic working group, which brings unbiased, diverse content expertise. Then, final drafts are shared with potentially biased but contrasting representative expert stakeholders for feedback and further refinement by the working group.

This research exemplifies how an empirical research strategy can directly inform the development of unbiased resources. This is an evolving model for identifying challenges and bridging knowledge gaps in a complex multi-stakeholder context.

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### Family Reference Samples, Missing Persons, Social Science

## A89 Practitioner Attitudes Toward the Management of Unidentified Human Remains in the United States

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**Learning Overview:** This research contributes to the investigation of policy and practice regarding Unidentified Human Remains (UHR) in the United States by examining factors that facilitate the management and identification of UHR cases.

**Impact Statement:** This presentation educates attendees about medicolegal practitioners' attitudes toward management of UHR—including awareness of laws, assessment of whether these laws are adequate, and degree of satisfaction with how agencies handle UHR cases—and what factors influence these perspectives.

Although identifying human remains is essential for legal, ethical, and humanitarian reasons, UHR cases pose a major ongoing challenge in the United States. In 2007, the National Institute of Justice declared the number of missing persons and unidentified dead a “silent mass disaster over time,” with the magnitude of the problem overwhelming local and state capacity to respond effectively.<sup>1</sup> Unlike mass disasters typically associated with sudden events, the missing and unidentified crisis in the United States spans decades. Between 1980 and 2004, for instance, the remains of approximately 10,300 UHR were reported to the United States National Death Index.<sup>2</sup> Currently, the remains of around 4,400 unidentified individuals are recovered annually in the United States, with roughly a quarter of those still unidentified after one year.<sup>3</sup>

State laws typically govern how UHR cases are handled. In the absence of such laws, institutional policy provides guidance. Because medicolegal practitioners are charged with upholding these laws and policies, they provide critical insight into understanding the challenges and issues related to the management and identification of UHR. Therefore, to assess the attitudes of practitioners toward the management of UHR cases, an online anonymous survey was disseminated to members of the American Academy of Forensic Sciences (AAFS) (Anthropology, General, and Pathology/Biology Sections); members of the American Board of Medicolegal Death Investigators (ABMDI); and Medical Examiners (MEs), coroners, and law-enforcement officials with publicly available contact details. In addition to professional demographic information, recipients were asked to comment on the adequacy of existing state laws and office policies regarding UHR and their awareness of and degree of satisfaction with these laws.

One hundred eighty-six individuals responded to the survey (AAFS  $n=85$ , AMBDI  $n=7$ , Other  $n=94$ ). Most responses (51.6%) were from coroners, followed by MEs (32.8%), sheriffs (4.8%), educational institutions and law-enforcement agencies (3.2% each), and other agencies (4.3%). Respondents whose offices provided training related to state policy for dealing with UHR were equally divided between yes and no (48.3% each), with 3.3% unsure. Almost twenty-five percent (24.7%) of respondents had worked for their current office or jurisdiction for 0–5 years, 20.4% for 6–10 years, and more than half (54.8%) for >10 years.

A two-way table with chi-square test indicated that training was significantly associated with awareness of laws regarding UHR ( $P=0.000$ ), assessments of adequacy of these laws ( $P=0.032$ ), and satisfaction with the ways in which offices handled UHR cases ( $P=0.020$ ). Respondents with training were more likely than expected to be aware of laws, find laws adequate, and be satisfied with laws, while respondents without training were more likely than expected to report themselves unaware of laws, find laws inadequate or be unsure about adequacy, and be occasionally unsatisfied or unsatisfied with handling of UHR cases. In addition, office type was a significant predictor ( $P=0.024$ ) of whether the respondent regarded laws regarding UHR as adequate. Respondents from coroners' offices were more likely to consider laws adequate, while respondents from MEs' offices were more likely to find laws inadequate.

These findings highlight the importance of training for medicolegal practitioners working with UHR across the United States and hint at fundamental organizational or professional differences underlying practitioners' perspectives. Results will be discussed in the broader context of policies regarding UHR.

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1. Ritter, N. Missing persons and unidentified remains: The nation's silent mass disaster. *National Institute of Justice Journal* 2007;(256):2–7). Hughes, K. I. Bureau of Justice Statistics, Fact Sheet, 2007. Report No.: NCJ 219533.
2. National Missing and Unidentified Persons System (NamUs). *The nation's silent mass disaster, 2021*. <https://www.namus.gov/> (accessed May 4, 2021).

### Unidentified Human Remains, Jurisdictional Authority, Medicolegal Practitioner

## A90 State Policy Regarding the Treatment of Unidentified Human Remains and the Need for Scientific Advocacy

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**Learning Overview:** Through this presentation, attendees will gain a better understanding of the complexities of state policies regarding the treatment of Unidentified Human Remains (UHR) in the United States.

**Impact Statement:** This presentation will impact the forensic science community by presenting data collected from all 50 states and Washington, D.C. regarding the treatment of UHR and highlighting the variability and inconsistencies in these state laws.

Investigating the identity of UHR who enter the medicolegal system is crucial for several reasons beyond criminal justice. These efforts enable families of the missing and disappeared to pursue restorative justice and seek closure through answers, and the reclamation of dignity and respect for the dead has been invoked globally in support of the imperative to identify the deceased.<sup>1-5</sup> Additionally, it is argued that universal, inherent rights apply not only to the living but also to the dead, and the failure to fulfill these obligations, the first of which is to establish identity, constitutes a human rights violation.<sup>4,5</sup> Despite these compelling factors, UHR cases continue to pose a major challenge for forensic practitioners, government officials, law enforcement, and local communities on a national and international scale.<sup>6</sup> To address the political, social, and structural barriers contributing to and resulting from this “silent mass disaster,” a community of practice that facilitates case resolution is desperately needed.<sup>6</sup>

This research examined policies from all 50 states and Washington, D.C. to better understand how UHR cases are being handled at the state level and to assess the impact of these laws on UHR casework. A series of questions ( $n=23$ ) regarding the investigative process, record keeping, and final disposition were evaluated using the most recent versions of state statutes publicly available through state legislative websites. The location of relevant laws relied on keyword searches including unidentified human remains, unknown decedent(s), missing person(s), autopsy, medical examiner, coroner, inquest, and DNA.

While 48 (94%) locales have active statutes relating to the treatment of UHR, only 32 (63%) explicitly state that investigation into the decedent's identity is required. Furthermore, only 29 (56%) specify postmortem data collection that could aid in the identification process and even fewer ( $n=19$ ; 37%) require genetic sampling when alternative means of identification are not productive. The paucity of comprehensive clearly stated, and organized policy regarding the treatment of UHR illustrates a potential cause for the continuation of the “silent mass disaster” defined by the National Institute of Justice in 2007.<sup>6</sup> However, even sound policy is inconsequential when not consistently applied in practice.<sup>7</sup> Future work will investigate the impact of United States Census Bureau state statistics (e.g., population size, GDP, poverty level, etc.) and UHR historic case statistics from the National Crime Information Center (NCIC) on policy regarding the treatment of UHR cases.

The unique perspective provided to anthropologists through specialized training within social and “hard” sciences enables forensic practitioners to take a more active role in the identification process beyond report generation and to serve their communities through scientific advocacy and working toward policy change. For instance, although many state policies discuss the treatment of UHR and the handling of missing persons cases, few states recognize that UHR and missing persons cases are often one and the same. This disconnect seems to be exacerbated within vulnerable populations, including people of color and transnational migrants. This study does not suggest that a “one-size-fits-all” approach is the most effective path forward in mitigating the overwhelming effects of UHR casework. However, by evaluating and understanding state policies, forensic practitioners may work toward crafting proactive, collaborative, and interdisciplinary solutions that will facilitate the herculean task of identification.

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1. G. Gaggioli. International Humanitarian Law: The legal framework for humanitarian forensic action. *Forensic science international* 282, 184-194 (2018).
2. G. Goad. Expanding Humanitarian Forensic Action: An Approach to US Cold Cases. *Forensic Anthropology* 3, 50-59 (2020).
3. S. Cordner; M. Tidball-Binz. Humanitarian forensic action—Its origins and future. *Forensic science international* 279, 65-71 (2017).
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5. Interpol General Assembly. 1996. *Disaster victim identification*. In Resolution No. AGN/65/RES/13. ICPO—Interpol General Assembly, 65<sup>th</sup> session.
6. N. Ritter. Identifying remains: Lessons learned from 9/11. *NIJ J* 256 (2007).
7. M.K. Spradley; N.P. Herrmann; C.B. Siegert; C.P. McDanel. Identifying migrant remains in South Texas: Policy and practice. *Forensic Sciences Research* 4, 60-68 (2019).

### Unidentified Human Remains, State Policy, Scientific Advocacy

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## A91 A Model for Multidisciplinary Identification Investigations in Medical Examiner and Coroner Offices: The New York City Office of Chief Medical Examiner (NYC OCME) Identification Review Committee

*Allison Sharplin, MA\*, New York City Office of the Chief Medical Examiner, New York, NY*

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**Learning Overview:** After attending this presentation, attendees will be familiar with the thorough decedent documentation protocols and investigative actions taken for decedents that are tentatively identified, unidentified, or that have complex alias issues leading up to presentation at the NYC OCME Identification Review Committee (IRC) meeting. The IRC applies a multidisciplinary quality assurance system for the confirmation of identity in the more challenging and complex cases, including decedents who are unknown without context, visually unrecognizable, and/or present with multiple identification aliases. These investigative methods and actions can be applied on a smaller or larger scale, depending on the need of the medical examiner or coroner office.

**Impact Statement:** This presentation will impact the forensic science community through the presentation of a multidisciplinary approach of forensic specialties to human identification in medical examiner and coroner offices. This model recommends thorough documentation of unidentified decedents as well as investigation into their identity and search for next of kin prior to temporary disposition.

The IRC format has its roots in Disaster Victim Identification (DVI) procedures in mass fatality management operations. Humanitarian law dictates that in natural disasters or armed conflict, the management of the dead is a priority during the response and survivors have a right to know the whereabouts and fate of their deceased loved ones. Common law also supports the right of next of kin and families to sepulcher (to choose and control the burial, cremation, or other final disposition of a deceased person). In day-to-day operations, medical examiners and coroners are tasked with confirming the identity of deceased persons in their care as part of the death investigation. A confirmed identification is necessary to notify the legal next of kin, resolve estate issues and criminal and/or civil litigation, and to issue death certificates.

At NYC OCME, decedents are brought into the office as unidentified or tentatively identified. Identifications are confirmed through modalities including visual, fingerprints, radiology, odontology, DNA analysis, or contextual methods. NYC OCME serves a large, diverse population that includes persons without close family, in a transient state, and otherwise without context. The steps leading up to case presentation at IRC ensure a thorough documentation of decedents, identification investigation, and next of kin search. Documentation of decedents begins on the day of autopsy or external examination. Identification Investigators work to confirm identifications. Family investigators search for next of kin.

As time passes from the date of death, decedents that remain unidentified or without resolution to complex aliases are presented to the IRC. The process of the IRC ensures an effective system to ensure a diligent search of available avenues for confirmation of identification, integration of several lines of evidence into a recommended identification outcome, as well as the search for next of kin for each decedent. The monthly IRC meeting consists of experts from each relevant OCME discipline who evaluate evidence presented and provide input for additional avenues of investigation: Identification Unit, Anthropology, DNA (Forensic Biology), Medicolegal Investigations, Medical Examiners, NYPD Missing Persons Unit, and Family Outreach. Each case discussed at IRC results in a recommendation to approve a confirmed identification through a selected modality, or additional investigative direction, recommendation for DNA analysis, or burial with an unverified identification status. After a recommendation of unidentified or unverified identification status from the IRC, temporary traceable burial in the city cemetery ensures that future methods can be utilized to confirm identification and allows future family members to determine final disposition.

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### **Decedent Identification, Unidentified Decedent, Alias**



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## A92 An Introduction to the International Committee of the Red Cross (ICRC) Resolve Platform, a Web-Based Application for the Management of Information of Missing Persons and Human Remains

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**Learning Overview:** After attending this presentation, attendees will have gained insights on the challenges, opportunities, and constraints of building an electronic tool for the management of information on missing persons and human remains.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new tool for the management of forensic data while also sharing experiences on the challenges and limitations encountered during its construction.

Through the ICRC's experience in multiple contexts, the ICRC has observed significant variation in methods and standards for the management of forensic data. Sudden surges in cases caused by disasters, armed conflicts, violence, and disease outbreaks further impede efforts to properly manage forensic data. As a result, traceability of unidentified and unclaimed remains may be lost, records may be unavailable or poor in quality, and, in some instances, remains may fail to maintain their identity in overwhelmed mortuaries, giving rise to administrative disappearances. Meanwhile, when systems and mechanisms are not in place for the proper management of information, family requests may be lost and families may not receive timely updates about the status of their cases.

To respond to modern challenges and assist both local missing persons agencies and mortuaries improve the management of information, the ICRC began building the Resolve Platform in 2017.<sup>1</sup> The overarching objective is to replace the existing tool developed in the 2000s (called AMPM Database) with a comprehensive solution that addresses the original needs, as well new challenges, such as managing large data sources, demand for remote collaboration, and integrating multiple sources of data of different quality.<sup>2</sup> The aim is to offer the application as an empty shell to requesting agencies to help improve management of information on missing and dead. The application can be utilized for the management of routine cases or cases resulting from emergencies by forensic specialists and non-specialists such as mortuary technicians and administrative staff as well as first responders.<sup>3</sup>

While there is growing need worldwide for such systems, there are also challenges when developing a technical platform that is flexible enough to be adapted to different operational environments, legal frameworks, languages, and workflows. Variations in local infrastructure and availability of human resources further compound efforts for the development of a solution. Similarly, there are added layers of complexity, such as building internal security requirements, storage availability, data protection standards, and the ability for software maintenance.

In late 2019, the ICRC completed the first module, which focuses on missing persons (antemortem data), events linked to disappearances, and potential burial sites. In late 2021, the second module was completed, which includes functionality for the data on recovery operations of sites and both field and lab documentation of human remains, as other cases (artifacts, samples).

This presentation will offer an opportunity to view key functions of the application with an emphasis on postmortem data while also sharing insights into the build process of the software.

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- <sup>2</sup> Hofmeister et al. The ICRC AM/PM Database: Challenges in forensic data management in the humanitarian sphere. *Forensic Science International*, Volume 279, October 2017, Pages 1-7.
- <sup>3</sup> M.S. Puerto; D. Abboud, J.P. Baraybar; A. Carracedo; S. Fonseca; W. Goodwin; P. Guyomarc'h; A. Jimenez; U. Krenzer; M.D. Morcillo Mendez; J.L. Prieto; J.R. Gonzalez; Y.R. Orozco; J. Taylor; A. Tennakoon; K. Winter; O. Finegan. The search process: Integrating the investigation and identification of missing and unidentified persons. *Forensic Science International: Synergy* (2021), doi: <https://doi.org/10.1016/j.fsisyn.2021.100154>.

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### Humanitarian Forensic Action, Human Identification, Forensic Data Management

## A93 An Overview of Recent Migrant Drowning Deaths in the Balkans

*Sherry Fox, PhD\*, International Committee of the Red Cross, Pristina, Kosovo*

**Learning Overview:** Migrants are drowning around the world, with families often suffering from the ambiguous loss of their loved ones. Although migrant drowning deaths in the Mediterranean Sea are generally known, attendees can expect to gain an appreciation for migrant drowning deaths on the European mainland in the Balkans, as migrants drown in rivers in their attempts to reach northern Europe. Secondly, unfortunately, some of these individuals are not identified nor will their identification be possible in the future.

**Impact Statement:** This presentation draws attention to migrant drowning deaths in the Balkans, particularly in rivers, as migrants attempt to reach northern Europe. Demographic information regarding sex and age data, when known, are presented, among other factors, including, for example, the times of year when migrant drowning deaths are occurring in the Balkans. Additionally, a shift in migratory patterns may be emerging, regarding the country of origin of migrants. Unfortunately, some dead migrants may never be identified, and as such, promotion of their dignified management is presented. It is hoped that by bringing attention to this situation, perhaps fewer migrant drowning deaths will occur in the future in the Balkans.

Migrant deaths along European maritime routes are well reported and are once again on the rise, with the Mediterranean leading the world among the deaths of migrants.<sup>1</sup> Many migrants passing through Greece and/or Turkey *en route* to northern Europe will traverse the Balkans. According to the International Organization for Migration (IOM) in 2021, through the month of July, the majority of migrants dying in Europe are dying in the Balkans by a factor of three times the combined deaths elsewhere on the continent.<sup>2</sup> The purpose of this study is to raise awareness of migrant drowning deaths in the Balkans. Utilizing data from the Regional Information Center (RIC) of the International Committee of the Red Cross (ICRC) collected for the purposes of helping family members locate their loved ones who have gone missing during migration, insight has been gained into recent migrant deaths in the region, including drowning deaths. It should be noted that along with the ICRC, the RIC data is gathered by many people among the various Red Cross societies as part of the Red Cross/Red Crescent Movement in the Balkans from published accounts where identity could not be established at the scene. Between February 24, 2015, and June 21, 2021, 95 incidents have been recorded in the RIC database documenting 166 deaths, including 59 drowning deaths or approximately one-third of migrant deaths in the Balkans. It should be noted that this figure is an underrepresentation of actual migrant drowning deaths in the region. Additionally, if bodies were not recovered from an incident, or if the cause of death of bodies recovered from bodies of water was unknown, these individuals were not recorded.

Information will be presented on migrant drowning deaths in the Balkans based on publicly available data, including: the number of deaths by drowning per incident, biological sex, age at death, month of drowning, where the drownings are occurring, country of origin, if these individuals are identified, and if the burial location is known. Results indicate that most migrant drowning deaths represent adult males, which is representative of the migrant population in the region. Most migrant drowning deaths in the Balkans are in rivers, including rivers demarcating political boundaries, such as the Danube. The largest number of migrants drowning per incident is nine, with two such incidents reported. The identities of these individuals often remain unknown, are from unknown countries of origin, and are with unknown burial locations. June and November are the deadliest months for drowning among migrants in the Balkans with no record of drowning deaths among migrants in the region during the winter months of January and February, and no record of drowning deaths among migrants in the summer months of July and August. The latter may be due in part to lower water levels in the smaller rivers during mid-to-late summer. There may also be a shift in recent years in the countries of origin among migrants with more migrants traveling from countries in Asia, such as Syria and Iraq, rather than from countries in Africa, as was common in the past. Additionally, burial locations of unidentified migrants need to be marked and recorded such that human remains can be exhumed if identified and ultimately returned to their loved ones.

The forensic sciences can contribute to helping solve humanitarian problems: by ensuring that the dignity of the deceased and the dignity of the bereaved are preserved; by restoring identities to the unidentified; by providing families of migrants with information on their loved ones; and by returning those identified to their families. It is hoped that by raising greater awareness of migrant drowning deaths in the Balkans, fewer migrants will die by drowning in the future.

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1. Cf. [https://missingmigrants.iom.int/Med\\_deaths\\_Jan-Jun\\_2021](https://missingmigrants.iom.int/Med_deaths_Jan-Jun_2021).
2. Ibid.

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### Migrant, Drowning, Balkans

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## A94 Forensic Crisis in Mexico: Lessons Learned From International Cooperation

*Aurora Marcela Pérez-Flórez, MSc\*, GIZ, Mexico City, Mexico; Maximilian Murck, MSc, Jr., GIZ, Mexico City, Mexico; María Fernanda Lazcano Campos, BSc, GIZ, Mexico City, Mexico*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the challenges of the forensic crisis in Mexico that has left more than 50,000 unidentified individuals in forensic institutes and public graves. Lessons learned from the perspective of international cooperation, especially between Mexico and Germany, will be presented.

**Impact Statement:** This presentation will impact and contribute to a technical and scientific discussion on how the international forensic science community can support international efforts to overcome this forensic crisis.

In Mexico, disappearances have been a common denominator since the late 1960s. Starting with the so-called “Dirty War” and even more so in the war against drug trafficking declared by former President Felipe Calderón, an important growth of this criminal phenomenon was identified. Today, Mexico has more than 90,840 missing persons according to the National Registry of Missing and Unidentified Persons (RNPNDNO) and around 52,000 Unidentified Deceased Persons (UDP).<sup>1,2</sup> Given the lack of action by the Mexican state, in 2009 groups of families of disappeared persons began to form throughout the national territory. These groups, together with the recent National Search Commission (CNB), have managed to locate hundreds of clandestine or official graves in all the states of the Republic. However, the identification processes do not advance at the same pace as problems associated with the lack of institutional capacities for identification, including the sparse use of fingerprints or DNA for identification purposes, deficient use of databases, lack of transparency, homologation of approved criteria, among others, shows that Mexico is currently experiencing a forensic crisis regarding human identification.<sup>2</sup>

This presentation collects the problems evidenced by international cooperation, especially the case of the German Development Cooperation (GIZ), a German government-owned development agency, with the project *Strengthening the Rule of Law* ([www.identificacionhumana.mx](http://www.identificacionhumana.mx)) in Mexico, and the learning and areas of opportunity that have been developed with a view to preventing this type of scenario in the future. The case of the State of Tamaulipas is presented, where a multidisciplinary forensic identification team with the support of the Forensic Anthropology Foundation of Guatemala (FAFG) and GIZ was created in the state attorney’s office. The implementation of process flows for the standardization of fingerprints will also be discussed. Likewise, the results of the generalized search processes (file searching) in mass graves of municipal cemeteries in Veracruz will be presented.

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### Human Identification, International Cooperation, GIZ

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## A95 Forensic Anthropology in the United Kingdom: The Evolving Landscape and Professional Certification of the Discipline

*Julie Roberts PhD\**, Liverpool John Moores University, Liverpool, United Kingdom; *Sarah Ellingham, PhD*, International Committee of the Red Cross, Geneva, Switzerland; *Rachael Carew, PhD*, Coventry University, Coventry, United Kingdom; *Linda Ainscough, MSc*, Cellmark Forensic Services, Chorley, United Kingdom

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**Learning Overview:** This presentation will outline the development of forensic anthropology and associated professional bodies in the United Kingdom. It will further discuss the positioning of forensic anthropology within the United Kingdom Criminal Justice System (UK CJS), the development of forensic anthropology degree programs, professional certification system and typical areas of employment, and types of cases commonly encountered in the United Kingdom.

**Impact Statement:** This presentation will impact the forensic science community by showcasing how the United Kingdom—as one of the forerunners in Europe—has professionalized the discipline of forensic anthropology, including the creation of a certification system and granting of chartered status to senior practitioners. This framework can function as a blueprint for countries who are yet to embark on professionalization of forensic anthropology.

Forensic anthropology first gained visibility in the United Kingdom in the 1990s through the involvement of United Kingdom Forensic anthropologists with the criminal tribunals for the former Yugoslavia and Rwanda, after which their assistance with domestic criminal cases was increasingly requested. Since the early 2000s, a number of United Kingdom universities have offered both undergraduate and postgraduate degrees in Forensic Anthropology, either alone or often paired with other fields. The establishment of a United Kingdom Forensic Science Regulator (FSR) in 2008 catalyzed the development of professional bodies in forensic anthropology, with the formation of the British Association for Forensic Anthropology (BAFA) and the Forensic Anthropology Committee of the Royal Anthropological Institute (RAI) in 2011. A collaboration between these two organizations sought to address the need for a national standardized framework for forensic anthropology practitioners in the United Kingdom. Further aims were to provide a career pathway for forensic anthropologists and a means of demonstrating professional competence.

In 2013, a three-tiered professional certification scheme was introduced by the RAI to provide a transparent framework for the practice of forensic anthropology casework in the United Kingdom, as well as transparency regarding competence for police, judiciary, and other clients. The framework further demonstrated a career development pathway through a system of mentorship and attaining competencies at each level of expertise. This United Kingdom certification scheme continues to evolve with the recent granting of Chartered status to those practitioners certified at the most senior level.

The forensic anthropology framework was developed in conjunction with the Forensic Science Regulator and was designed to meet the evolving quality standards required in the United Kingdom CJS. Importantly, all United Kingdom forensic science practitioners are now required to adhere to the FSR Code of Practice and Conduct, and forensic anthropologists are required to practice in compliance with the RAI Code of Practice for Forensic Anthropology published in 2018. Further, the Forensic Science Regulator was granted statutory powers in May 2021, meaning that quality standards can now be legally enforced.

This presentation highlights developments in forensic anthropology that have been made in the United Kingdom over the past 30 years in education, policy, and practice. Future challenges and goals around continuing to evolve with the modern forensic science world will also be discussed, including the extension of the certification scheme to internationally based forensic anthropologists, the impact of the FSR gaining statutory powers, and concerns regarding the limited accessibility to casework to gain practical experience in the United Kingdom.

The goal of this presentation is to highlight this successful example of how forensic anthropology in the United Kingdom has been transformed from an almost unknown discipline to a fully recognized profession in its own right. The framework presented can serve as a blueprint for countries who are yet to embark on their journey of professionalization of the discipline and facilitate the continued evolution of professional forensic anthropology practices globally.

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### Forensic Anthropology, Professionalization, United Kingdom

## **A96 Forensic Anthropology Casework From Switzerland (Bern): A Ten-Year Survey**

*Sandra Lösch, PhD\*, Institute of Forensic Medicine, University of Bern, Bern, Switzerland; Lara Indra, MSc, Institute of Forensic Medicine, University of Bern, Bern, Switzerland*

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**WITHDRAWN**

**A97 The Importance of an Integral Approach to Recovery and Analysis of Commingled Mass Graves: The Koricanske Stijene Example**

*Almir Olovicic, MSc, International Commission on Missing Persons, Sarajevo, BiH, Bosnia and Herzegovina; Sandra Sostaric, MA\*, International Commission on Missing Persons, Sarajevo, BiH, Bosnia and Herzegovina; Bojana Tomasevic, BSc, International Commission on Missing Persons, Sarajevo, BiH, Bosnia and Herzegovina; Beisa Talic, BSc, International Commission on Missing Persons, Sarajevo, BiH, Bosnia and Herzegovina; Samra Terzic MA, International Commission on Missing Persons, Sarajevo, BiH, Bosnia and Herzegovina*

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**WITHDRAWN**

## A98 Education and the Biological Profile: Survey Data on the Construction of the Biological Profile

*Ashley Smith MSc\*, Universit of Toronto, Toronto, Ontario, Canada; Alexis Dzubak, MS, The Ohio State University, Columbus, OH; Alexandra Saly, MSc, University of Toronto, Toronto, Ontario, Canada; Evonne Turner-Byfield, MS, The Ohio State University, Columbus, OH; Justin Maiers, MS, University of South Florida, Tampa, FL*

**Learning Overview:** After attending this presentation, attendees will have learned about the congruences and the variability that exist in the process of learning to construct a biological profile, as well as the perceived efficacy of the practice of constructing the biological profile in practice.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the pedagogical practices taught across different universities and subdisciplines of anthropology and how they affect the construction of the biological profile.

The four primary biological profile categories are used to develop a potential identity of a set of skeletal remains and are based on metric and morphometric data. Recently, the efficacy of the biological profile has been questioned. The potential for different results highlights the necessity for education and practice, yet this type of training is not standardized from institution to institution. With the variety of interconnected external variables that influence ancestry assessments, small differences in the education and experience of the observers may result in dramatically different outcomes in final reports. Furthermore, the range of human variation found in any population is perpetually in flux, which makes reflection on the techniques used in ancestry assessment critically important. Likewise, sex estimation brings along its own social and cultural variations, particularly with regard to transgender, non-binary, and gender non-conforming individuals. This means that an individual in a given case may have identified with a different gender than what may be recorded using current anthropological practices and procedures.

Beyond the social aspects of the biological profile, other aspects of the profile also demonstrate questions as to “usefulness,” namely that of age and stature estimations. Adult age estimation methods as currently used and taught tend to have wide ranges, with some demonstrating statistically valid ranges (95% Confidence Interval [CI]) of  $\pm 15$ –20 years or more. Further complicating matters is the fact that morphological traits, both macroscopic and microscopic, can be affected by factors such as genetics, pathology, and even trauma. One of the greatest problems for stature estimation is errors in how measurements are taken, resulting in actual measurement error. Further, the resulting stature ranges emanating from the calculations tend to be wide themselves, ranging around  $\pm 5$ ", which could potentially cover more than a plurality of the population's average stated stature.

With these in mind, a study was conducted on the educational practices, utility, and efficacy of the biological profile, both in graduate and undergraduate institutions, as well as with practicing professionals. An anonymized study was conducted and sent out to 80 academic institutions, as well as selected professional organizations and listservs. The survey was hierarchical in nature with similar questions posed to both students and educators/professionals, while others were geared toward one group or another. There was a mix of quantitative and qualitative questions with categories including educational background/career focus, training in the construction of the biological profile, importance of the profile in anthropological casework, and reflections on individuals' practice in the field. Analysis was limited to basic descriptive statistics regarding the quantitative data and thematic analysis for the qualitative data.

While data collection is on-going, preliminary data was collected from a number of students and suggests they view forensic anthropology as primarily consisting of the biological profile while also reporting a lack of hands-on experience requiring looking outside their home institutions for mentorship and training. Further, students noted an interest in more training in taphonomy and trauma to augment the biological profile and that course textbooks are not sufficient in providing a comprehensive understanding of the field. In addition to the survey, anecdotal results demonstrated there is a variance in the training and understanding of the biological profile, in both practice and theory.

Given the results, this study has compiled a series of recommendations to the field including, but not limited to: the creation of a better textbook for undergraduate and graduate training; the incorporation of tangent fields, such as statistics and theory, as a part of required coursework; the creation and use of diversified skeletal teaching collections' and, for graduate education, training in an applied setting.

### Biological Profile, Pedagogy, Praxis

## A99 Theoretical and Methodological Considerations of the Estimation of Osteological Sex Utilizing Fuzzy Rule-Based Inference Systems

Katherine Lane, MA\*, University of Central Florida, Orlando, FL; Donovan Adams, PhD, University of Central Florida, Orlando, FL

**Learning Overview:** The goal of this presentation is to inform attendees of the variation in biological sex, which is not discrete and binary as traditionally conceived and constructed within forensic anthropology, and how this information may be incorporated into quantitative models.

**Impact Statement:** This research will impact the forensic science community by rethinking how sex is framed and estimated in forensic anthropology by examining how individuals cluster and are classified using fuzzy logic.

Forensic anthropologists traditionally estimate sex in a binary, discrete model that does not reflect biological sex variation. Forensic anthropologists differentiate between gender and biological sex, with biological sex being assigned at birth primarily on the basis of external organs and impacted by a combination of chromosomal, hormonal, and anatomical characteristics. The biological variation for each of these factors exists on a spectrum of their own, and there can be a discordance between these aspects of sex.<sup>1</sup> From this, the estimation of sex in forensic anthropology is not as clear-cut as traditionally considered, and focusing on only a two-sex model reinforces biological normalcy.<sup>2,3</sup>

This study investigates the variation in biological or, more specifically, osteological sex using craniometric data of females ( $n=961$ ) and males ( $n=1,634$ ) from the Forensic Data Bank to address: (1) do the categories of female and male accurately reflect biological variation? and (2) how do fuzzy principles affect classification in our current binary system?<sup>4</sup>

Descriptive statistics and exploratory tests were conducted for both sexes. Fuzzy C-Means (FCM) was conducted on nine dimensions derived from principal component analysis on a reduced (to limit the amount of estimated data) and k-nearest neighbor ( $k=5$ ) imputed data set to examine patterns within the data and estimate the optimal number of clusters.<sup>5-7</sup>

The FCM analysis resulted in two optimal clusters being defined with a fuzzifier of 1.3. While the FCM produced a two-cluster model, the degree of membership to each cluster and the cluster of maximum membership varied for both males and females, though female clustering results exhibited a narrower range of variation than males.

Two fuzzy classification methods were tested on the data to approximate traditional forensic methods.<sup>8</sup> These included Chi's (FRBCS.CHI) and rule-weighted (FRBCS.W) fuzzy rule-based classification systems. Additionally, Adaptive-Network-based Fuzzy Inference System (ANFIS) was tested as a regression model.<sup>8</sup> The FRBCS.W and FRBCS.CHI with 12 Gaussian membership functions developed on the training model was found to have the highest "accuracy" rate into traditional sex categories (FRBCS.W—76.62%; male: 86.98%, female: 57.98%; FRBCS.CHI—76.43%, male: 82.54%, female: 65.43%). These methods were also tested on three clusters derived from the FCM analysis to assess the impact of expanding from the traditional two-sex model on estimating osteological sex. For the three cluster models, the FRBCS.W (76.81%) and FRBCS.CHI (76.24%) with ten Gaussian membership functions were found to have the highest "accuracy" rate. ANFIS models generally showed a grouping of individuals into one category or cluster; however, additional research will investigate the impact of type and number of membership functions in the utility of these models.

These preliminary results indicate substantial overlap between traditional categories of male and female and that these binary categories fail to capture the variation observed in biological sex. There is potential to continue exploring the utility of fuzzy techniques to address issues of bionormalcy in the discipline. By using unsupervised clustering and allowing for multiple memberships, it is possible to observe natural, rather than constructed, patterns in the data that may be used to better inform practice/research.

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### Fuzzy Rule-Based Inference Systems, Osteological Sex, Sex Estimation



## A100 The Precision of Morphoscopic Traits for Sex and Ancestry Estimation From 3D Surface Scans of the Crania

Sarah Schwing, MA, University of Tennessee-Knoxville, Knoxville, TN

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the utility of virtual human skeletal elements as proxies to physical bone in the construction of the biological profile. This presentation will focus on the precision and reliability of morphoscopic data for sex and ancestry estimation obtained from 3D surface scans of crania.

**Impact Statement:** This presentation will impact the forensic science community by discussing a novel application of two widely used morphoscopic methods, Walker's cranial traits for sex estimation and Hefner and Ousley's traits for the Optimized Summed Scored Attributes (OSSA) method for ancestry estimation, to virtual human skeletal remains.<sup>1,2</sup>

Virtual anthropological investigations of morphoscopic traits have increased over the past two decades, as virtual approaches have gained traction within forensic anthropology.<sup>3</sup> The majority of published studies focusing on morphoscopic traits, however, pertain to the Suchey-Brooks age estimation method using the pubic symphysis.<sup>4</sup> Few additional skeletal elements or biological parameters (e.g., sex or ancestry) have been considered in such morphoscopic investigations and, to date, it appears no virtual anthropological investigation has focused on the Walker or OSSA morphoscopic methods for sex and ancestry estimation.

Further, most virtual anthropological investigations have employed Computed Tomography (CT) technologies. As such, research regarding the precision of morphoscopic data obtained from 3D surface scans is scant and error rates for these virtual methods are unknown, making said techniques inadmissible in courts of law under the *Daubert* criteria.<sup>5</sup>

This investigation sought to understand the applicability of methods developed for use on dry bone to virtual models, specifically 3D surface scans, by analyzing the agreement between morphoscopic data obtained from paired dry and virtual skeletal elements. Using a sample of 42 crania sourced from the William M. Bass Donated Skeletal Collection, housed at the University of Tennessee, Knoxville, Walker's cranial traits for sex estimation and OSSA cranial traits for ancestry estimation were evaluated for agreement across dry and virtual human crania, serving to inform the precision of virtual evaluation of these traits. The impact of experience on inter-format score agreement (e.g., dry scores vs. virtual scores) was also investigated through the inclusion of six additional scorers of divergent experience levels. These results also offer a more informed understanding of the precision and ease of implementation of these methods virtually.

Weighted Kappa tests revealed slightly higher agreement between dry and virtual formats among Walker traits than OSSA traits, with inter-format comparisons of the former demonstrating fair to almost perfect agreement (Kappa values=0.277–0.834), and the latter demonstrating slight to substantial agreement (Kappa values=0.159–0.720). Results also suggest that graduate versus professional experience does not have a statistically significant impact on score agreement between dry and virtual formats.

This study demonstrates the potential for 3D surface scans, and virtual human skeletal elements in general, to serve as proxies for physical bone in research and casework when the latter are unavailable or inaccessible. This research also helps lay the foundation for modifying traditional morphoscopic methods to best suit virtual evaluation of human skeletal remains.

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### 3D Surface Scans, Morphoscopic Traits, Virtual Anthropology

## **A101 An Evaluation of the Influence of Socio-Cultural Identity on the Morphology of the Mid-Face Among Black South African Groups for Forensic Implications Using 3D Surface Scanning Modality**

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**Learning Overview:** After attending this presentation, attendees will better understand the influence of Black South African socio-cultural identity on variation in the shape of the skeletal mid-face.

**Impact Statement:** This presentation will impact the forensic science community by providing data on mid-facial shape configurations and on sub-specific discriminate shape matrices for South African socio-cultural identity estimation.

Due to the high number of unidentified bodies in Gauteng province each year, researchers have developed South African standards for establishing a biological profile to identify unknown persons.<sup>1</sup> However, as significant differences in population affinity-related morphological variations exist, the probable identification of an unknown individual is based on the presence of quantifiable phenotypic variations and the relationship of these variations to the individual's socio-cultural identity (e.g., Khoisan, Pedi, Sotho, Swazi, Tsonga, Tswana, Venda, and Zulu).<sup>1</sup> This study aims to evaluate the influence of socio-cultural identity on the morphology of the mid-face among a Black South African sample, with a particular emphasis on developing standards for predicting mid-facial variation within this population.

The sample consisted of 120 adult South Africans representing eight Black South African socio-cultural groups, namely Khoisan, Pedi, Sotho, Swazi, Tsonga, Tswana, Venda, and Zulu, who were obtained from the Pretoria Bone Collection in the Department of Anatomy, at the University of Pretoria.<sup>2</sup> 3D modeling of the relevant anatomical area was performed using a NextEngine® 3D surface scanner Ultra HD. The 3D anatomical extraction was performed by placing standard craniometric landmarks and sliding landmarks (interpolation factor=1mm) on 3D models using the Avizo® 9.4 software. Forty-one craniometric and 378 sliding landmarks were recorded on 3D models. A reproducibility testing of the landmarks and sliding landmarks on these 3D models were assessed and calculated using the dispersion analysis. A socio-cultural groups analysis on all shape matrices acquired was visualized and quantified through applying Geometric Morphometric Methods (GMM). All shape configurations (e.g., Mid-Face Landmarks (MFL) (18), Mid-Face Sliding Landmarks (MFLS) (378), as well as sub-specific discriminate shape matrices (e.g., Nasal Aperture Sliding Landmarks [NASL]) (50); Nasal Bones Sliding Landmarks (NASL) (16); right and left Zygomatic Sliding Landmarks (ZSL) (curve 1=21; curve 2=22; and curve 3=24); and right and left Maxillae Sliding Landmarks (MSL) (curve 1=29; curve 2=25; and curve 3=30), were analyzed.

Both anatomical and sliding landmarks were reproducible (<2mm). The analysis of variance showed that variation in midfacial shape were statistically significant ( $p < 0.001$ ) for all eight socio-cultural groups for all configurations, including sub-specific discriminate shape matrices, separately. Additionally, cross-validated linear discriminant function analysis yielded an accuracy between 79.59% and 100% for all shape configurations, as well as sub-specific discriminate shape matrices (MFL=93.10%; MFLS=97.96%; NASL=85.71%; NBSL=95.92%; right ZSL=83.67% (curve1), 100% (curve2), 85.71% (curve3); left ZSL=94.90% (curve1), 79.59% (curve2), 90.82% (curve3); right maxilla=83.67% (curve1), 97.96% (curve2), 93.88% (curve3); left maxilla=83.67% (curve1), 92.86% (curve2), 96.94% (curve3) reflecting the discriminative power of socio-cultural groups in the Black South African population.

In contrast to classical non-metric and metric approaches based on morphological traits, linear distances, and angles, the utilization of geometric morphometric for socio-cultural estimation using the midface retain the objects' geometry and analyses subtle differences among structures. In forensic analysis, the use of the geometric morphometric overcomes the non-utilization of fragmented bones by creating sub-specific discriminate shape matrices to analyze specific patterns not readily observable with traditional approaches. In a real-life application, reliable, accessible, and more efficient methods using 3D imaging approaches may allow forensic anthropologists to estimate the socio-cultural identity of Black South Africans in a more precise and repeatable way.

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### **Skeletal Mid-Face, Sub-Specific Discriminate Shape Matrices, Geometric Morphometrics**

## A102 Assessing the Validity of Palate Shape as an Indicator of Population Affinity Through the use of Elliptical Fourier Analysis (EFA)

Christopher Goden, MA\*, The Ohio State University, Columbus, OH; Alice Gooding, PhD, Kennesaw State University, Kennesaw, GA

**Learning Overview:** The learning objectives for this presentation will allow attendees to: (1) better understand the analysis of macroscopic traits through a contour-fitting geometric morphometric approach, EFA; and (2) enable a better understanding of the limitations associated with the use of EFA in relation to palate shape.

**Impact Statement:** This presentation will impact the forensic science community by quantifying palate shape using a geometric-morphometric approach. Additionally, this presentation will provide new information on human skeletal variation of palate shape while also assessing its validity in relation to the estimation of population affinity.

The estimation of population affinity from skeletal remains has become a highly debated topic within the forensic science community, largely attributed to the macroscopic methodologies employed by forensic anthropologists when assessing the craniofacial complex. While quantitative methodologies exist for the estimation of population affinity, forensic anthropologists employ macroscopic trait observation of the craniofacial region argued to be experienced-based and lack statistical backing.<sup>1</sup> As a result, it is feared by some that improper use of observation methods may hinder identification efforts of skeletal remains.<sup>1</sup> Conversely, others argue that the macroscopic trait analysis is reliable and replicable with proper training.<sup>3</sup> Prior research has demonstrated the utility of EFA to accurately quantify complex closed-contour shapes and uses resulting in data to delineate population affinity of skeletal remains.<sup>2</sup> However, at present, many traits commonly assessed via macroscopic observation (e.g., palate shape) have not been evaluated with EFA.

In response to the call to action for empirical assessment of macroscopic trait observation,<sup>1</sup> this pilot study quantifies the shape of the palate using EFA to explore the variation present among three modern population groups. Further, this study evaluates classification rates of population affinity with resulting EFA data.

3D craniofacial volume renderings were constructed from postmortem Computed Tomography (CT) scans of 150 individuals of equally represented Black, Hispanic, and White population groups obtained from the New Mexico Decedent Imaging Database (75 males, 75 females). Once visualized in a standardized orientation, 2D images of the palate were obtained, outlined in Adobe® Photoshop®, and subjected to EFA using SHAPE 1.3 software. Following obtainment of EFA coefficient and Principal Component (PC) data, Analysis of Variance (ANOVAs) were performed to assess for variation among the three population groups and between sexes. Linear Discriminant Function Analyses (DFAs) and Bootstrap Random Forest Modeling (RFM) were utilized to evaluate correct classification rates of population groups. It was hypothesized that correct classifications would meet or exceed 85% correct classifications.

Principal Component Analysis (PCA) of reconstructed contours exemplifies similar amounts of variation within and among all three population groups regardless of sex. ANOVA results indicate insignificant amounts of variation between sexes and among the three groups ( $p>0.05$ ). DFAs performed demonstrate correct classification rates varying from low classifications (23% for all groups) to moderately successful (73% for Black and White groups independent of sex). Moreover, RFM exhibits 87% correct classifications for all groups.

Results demonstrate that EFA is useful in understanding variation of palate shape within and among groups. Nonetheless, forensic anthropologists should use caution when utilizing statistical methodologies in conjunction with EFA as discrepancies exist when comparing different statistical classification analyses. Specifically, in this study, DFA performed with lower correct classifications when compared to RFM. Results of DFA indicate that the palate does not confidently delineate population affinity. Additionally, results of DFA may mask some individualizing expression exhibited between individuals. Conversely, results of bootstrap RFM indicate highly successful classifications, suggesting that machine learning techniques, such as RFM, may be of better use with EFA data.

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### Forensic Anthropology, Population Affinity, Palate Shape

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## A103 A Survey of Ancestry Estimation Method Preferences and Utilization in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of which ancestry estimation techniques and methods are most preferred and most frequently utilized by practicing forensic anthropologists.

**Impact Statement:** This presentation will impact the forensic science community by presenting the findings from a survey that sought to identify the most preferred and the most frequently utilized ancestry estimation techniques and methods in forensic anthropology.

The published literature contains conflicting statements regarding which ancestry estimation methods are the most preferred and employed by practicing forensic anthropologists. However, to date, there is no published research that examines the preferences and employment of ancestry estimation methods by forensic anthropology practitioners. To assure that further research into ancestry estimation is applicable to practicing forensic anthropologists, it is important to know which methods are preferred and utilized most frequently.

In this study, an anonymous online survey was developed to determine the most preferred and most frequently utilized ancestry estimation methods and techniques among forensic anthropologists and other disciplines that similarly examine human skeletal remains. Of the 109 respondents, 67% had completed or were in the process of completing a doctoral degree, with a mean year of degree completion of 2013. Approximately 40% of respondents were trained in the United States, 40% in the United Kingdom, and the remainder were trained elsewhere, predominantly in Europe. Of all respondents, 56% reported that they had forensic casework experience.

While the words “technique” and “method” are often used interchangeably, within this study the two words have different, non-interchangeable meanings. “Technique” refers to a *general* process or procedure of estimating the ancestry of skeletal remains. In this study, three different techniques were considered: the type of assessment (e.g., metric, non-metric); the skeletal element examined (e.g., the cranium); and the type of analysis (e.g., trait list, computer software program). “Method” refers to a *specific* process or procedure of estimating the ancestry of skeletal remains (e.g., the Rhine (1990) method, FORDISC®).

Preliminary analysis of the results revealed that among all respondents, the most preferred techniques to estimate ancestry were a combination metric and non-metric assessment, a cranial examination, and a trait list analysis. Additionally, the results revealed that among all respondents, the most preferred method to estimate ancestry was FORDISC®. These findings align with the results of the most utilized techniques and method by practicing forensic anthropologists.

Of the 56% of respondents who reported forensic casework experience, the most utilized assessment technique was a combination of metric and non-metric. The most examined skeletal element was the cranium, and the most utilized analysis technique was a trait list. Additionally, of respondents with casework experience, the most utilized method to estimate ancestry was FORDISC®. When respondents were asked why they utilize these techniques and this method in forensic casework, the most selected reason was that the techniques and method are perceived as accurate.

These insights into the most preferred and most utilized techniques and methods can help to ensure that further research into ancestry estimation is applicable to the practicing forensic anthropologist. The results of this study can be used to further inform future research into the most utilized ancestry estimation techniques, can assist in the development of comparative studies focusing on several of the most utilized methods, or can explore new approaches that can complement the currently utilized methods.

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**Ancestry Estimation, Forensic Anthropology, Forensic Science**

## A104 A Pilot Study to Improve the Identification of Unknown American Indians and Hispanic/Latinx Americans

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**Learning Overview:** Attendees of this presentation can expect to learn about the limitations currently facing forensic anthropology in contributing to dismantling the epidemic of missing American Indians (AI). Further, attendees will learn about ongoing research to improve identification of AI and Hispanic/LatinX Americans (HLA).

**Impact Statement:** This project will impact the forensic science community by making important improvements to the Forensic Databank and FORDISC®, a common tool for forensic anthropologists estimating population affinity, by increasing sample sizes for underrepresented groups

AI are disproportionately affected by the crisis of unidentified decedents; there are more than 4.5 times more missing AI reported than human remains that have been found and estimated to be AI.<sup>1-3</sup> One reason for the large number of missing AI may be the misclassification of their remains as some group other than AI. Misclassification greatly reduces the likelihood that a missing person can be matched with human remains that have been recovered. The pilot study described here demonstrates the use of up-to-date methods coupled with a new research resource, the New Mexico Decedent Image Database, to improve the accuracy of population affinity estimation for AI and HLA.<sup>4</sup>

Pilot data include 3D craniometric coordinate data (52 points) from 53 AI and 10 New Mexican HLA from a Computed Tomography (CT) dataset. These data were compared with the same data points collected from crania of migrants to the United States from Mexico, Guatemalan Mayans, and Yucatecan Mayans ( $n=181$ ). Both Mahalanobis and Procrustes distances between group centroids were significant for all pairwise comparisons. This preliminary research indicates that: (1) it should be possible to accurately estimate population affinity for AI with the sample proposed here; and (2) there is a great deal of variation within the sample this study intends to collect. This second observation provides hope that, even though this sample is limited to AI from New Mexico and eastern Arizona, it may be useful across a wider geographic area. Also, although the sample is small, New Mexican HLA are generally different from the AI sample and at the extreme of the Latin American samples. While it seems very likely that it will be possible to discern AI from HLA with the data this study intends to collect, more data are needed to determine whether it will be possible to reliably estimate whether an unknown individual was a United States citizen or a migrant, for example, New Mexican or Mexican.

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### Population Affiliation Estimation, Computed Tomography (CT), Craniometrics

## A105 Postmortem Radiological Computed Tomography (CT) Identification Based on Cranial Sutures

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**Learning Overview:** After attending this presentation, attendees will better understand the application of Sekharan's method for human identification using X-rays of cranial sutures on Computed Tomography (CT) scans. This is a preliminary study.

**Impact Statement:** Attendees will be informed of an improved and advanced identification method using head CT scans for positive human identification based on cranial suture morphology. With the increased usage of CT scans, both in postmortem examination in forensic facilities and in medical treatment, their usage for identification will become increasingly relevant.

**Background:** One of the methods for determining positive personal identification is the comparison of postmortem radiological images of decedents and antemortem radiological images of their presumed identity. Cranial suture morphology is permanent throughout life from the age of seven and usually remains visible into the seventh decade.<sup>1,2</sup> Although cranial suture morphology is considered unique, research on its use for identification is scarce.<sup>3</sup> In 1985 and 1989, Sekharan studied 320 skulls and 8,000 skull radiographs and determined that suture patterns constitute positive means of identifying the person, providing that there are antemortem records available for comparison.<sup>3,4</sup> In 2004, Rogers and Allard reviewed Sekharan's methodology in the framework of expert testimony and the *Daubert* ruling.<sup>1</sup> They argue that the method as it is originally described encounters problems such as lack of pattern description and recognition and the necessity for the antemortem and postmortem radiographs to be obtained from the same angle. Due to an increase in antemortem CT scans, performing postmortem CT scans at forensic institutions may become of paramount importance in cases of confirming identification as they can reveal significant details of cranial anatomy that can be used for identification.<sup>5</sup>

**Goals:** (1) To examine the applicability of Sekharan's radiograph method of using cranial suture morphology for postmortem CT scans, and (2) to develop an improved and suitable method for using cranial suture morphology for personal identification.

**Materials and Method:** Three hundred postmortem head CT scans (150 males, 150 females; age range 10–92) from the National Center of Forensic Medicine, Israel, were used for this study. According to Sekharan's method, eight areas on the sutures were examined, two on the sagittal and three on each side of the lambdoid. The effect of age and sex on the identification of suture patterns was also examined. Subsequently, 30 antemortem and postmortem paired scans from the same individuals were also examined in order to confirm that the same pattern could be identified in both.

**Results:** Sekharan's method for identifying suture morphology on radiographs was found to be not applicable to CT scans. In this study, Sekharan's ten patterns were reduced to four different morphological patterns of the suture: (1) Single Direction; (2) Wavy; (3) Closed; and (4) Complicated. These morphological patterns were common and easily identified in both sexes on 41% ( $n=123$ ) of the CT scans that were examined. In the other 59% of the scans, no clear pattern could be identified. These four suture patterns were most identifiable adjacent to the meeting point of the sagittal and lambdoid sutures ( $\lambda$ ). The Wavy pattern was found to be the most common pattern, hence the least indicative pattern for identification. The Closed pattern was found to be the least common pattern, hence the most indicative pattern for identification. The same variations of patterns could be identified in both antemortem and postmortem paired scans.

**Conclusion:** Based on this preliminary study, suture morphology can be used for personal identification using CT scans.

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### Cranial Sutures, Identification, Computed Tomography (CT)

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## **A106 A Multifaceted Short Tandem Repeat (STR) and Next Generation Sequencing (NGS) Assessment of Burned Human Remains Using Comparative DNA Extraction and In-Solution Hybridization Capture**

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**Learning Overview:** Attendees will leave this presentation with a better understanding of how fire impacts the preservation of DNA in human bones and teeth.

**Impact Statement:** This research impacts the forensic community by presenting DNA evidence obtained from victims of fire-related incidents from Maricopa County, AZ.

DNA analysis is an important tool in the identification of human remains recovered from forensic contexts. Under ideal conditions, DNA is sufficiently preserved for rudimentary Polymerase Chain Reaction (PCR) -based analysis, including STR profiling. However, exogenous factors such as depositional environment, microbial activity, and time since death often limit the amount of recoverable DNA from skeletal material. In circumstances where skeletal tissues are exposed to more severe insults, such as fire, obtaining adequate quantities of DNA for downstream analysis has proven even more challenging. Recent studies have shown that DNA yields are inversely proportional to the degree of thermal alteration of skeletal tissues. The amount of DNA obtained from bones and teeth are primarily impacted by the temperature and duration of fire exposure. To better understand how fire impacts DNA preservation, this study employed a suite of traditional and NGS analyses to assess a range of burned human remains. More specifically, this study compares quantitative PCR, STR, whole-mitochondrial genome, and genome-wide Single Nucleotide Polymorphism (SNP) results obtained from real-time fire victims from Maricopa County, AZ, using comparative ancient and forensic DNA extraction methods coupled with in-solution targeted enrichment techniques.

A total of 27 bone and tooth samples were transferred from the Maricopa County Office of the Medical Examiner (MCOME) for DNA analyses at the Molecular Anthropology Laboratory at Arizona State University. Bone and tooth samples were further subsampled into five burn categories. This study extracted DNA from these burned victims using two different DNA extraction methods: one devised by Loreille and colleagues (forensic DNA) and a second using a modified Dabney and colleagues (ancient DNA) extraction protocol. DNA concentrations were measured using the Quantifiler™ Trio DNA Quantification Kit. STR profiles were generated using the Promega® PowerPlex® ESX 17 Fast System, a kit that co-amplifies 16 STR loci, as well as the sex-typing amelogenin marker. In addition, this study re-extracted and built double-stranded DNA libraries from the burned remains, then enriched those libraries for whole mitochondrial genomes and genome-wide SNPs (5k panel) using synthetic biotinylated baits. Libraries were pooled and sequenced Illumina® MiSeq® and NextSeq® 550 platforms and the resulting reads were processed using an in-house custom computational pipeline. The analysis suggests that both extraction methods are suitable for obtaining DNA yields for STR and NGS analyses at lower temperatures. In the case of STR analysis, the ancient DNA extracted samples produced higher quantities for STR calling at temperatures >350°C. These results also suggest a critical point of DNA degradation at temperatures >350°C, wherein a significant drop in STR alleles, mitochondrial DNA (mtDNA) read counts and depth of coverage, and SNP calls are observed. The integration and adoption of optimized ancient DNA protocols, such as Dabney and colleague's DNA extraction protocol, in addition to in-solution targeted capture methods offers an alternative means to study DNA preservation in skeletal tissues subjected to a high degree of thermal alteration and pyrolytic degradation.

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### **Forensic Genomics, Ancient DNA, Burned Human Remains**

## A107 Centering Gender-Inclusive Strategies in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will understand the importance of gender-inclusive strategies in forensic anthropology and will learn how to center these in casework and research.

**Impact Statement:** Gender inclusive strategies are needed to improve case resolution for Transgender and Gender Variant (TGV) decedents, prevent further postmortem marginalization of vulnerable individuals, and promote diversity and inclusion in the forensic sciences. This presentation impacts the forensic science community by offering suggestions for incorporating gender-inclusive strategies in casework and scholarship.

Fatal violence against TGV individuals in the United States has been characterized as an epidemic.<sup>1</sup> At this time, 2021 is on track to be the deadliest year on record for TGV individuals.<sup>2</sup> Cultural attitudes toward the TGV population can hinder identification efforts and further marginalize TGV decedents through underreporting and non-standard documentation of missing and murdered persons, inequitable distribution of investigative resources, inaccurate and inconsistent reporting of gender, (dead)names, and sex, and unfamiliarity with context clues related to gender identity.<sup>3</sup>

The following are suggestions for how anthropologists can contribute to the development of gender-inclusive strategies in education, research, and casework that recognize and address the social factors impeding identification of TGV Does.

**Prioritize the needs and concerns of the TGV community.** Researchers should account for the safety and privacy of research participants, engage with community members as collaborators, and pursue research questions informed by the wants and needs of community members. Adams et al. provide guidelines for transgender health research focused on collaboration, language, accountability, informed consent, protection, allyship, and censorship; these can also guide forensic anthropology research and casework.<sup>4</sup>

**Engage with social theory and incorporate it into casework and research.** Queer theory critiques essentialist views of sex and gender, therefore exhibiting how Westernized perceptions of sex and gender are assigned to the skeleton. Additionally, intersectionality and structural vulnerability frameworks illustrate why Black and Latinx transwomen and Two Spirit Indigenous people are at the highest risk for lethal violence.

**Commit to education and continued conversation.** Educating oneself on issues of gender diversity, violence against TGV individuals, and current literature in and out of forensic anthropology is the first step to supporting this at-risk population. Tallman and colleagues found that only 42.4% of forensic anthropologists surveyed view sex as non-binary, and 75% were unfamiliar with gender-affirming surgeries, indicating that intra-field conversations about sex and gender are necessary.<sup>5</sup> Anthropologists should incorporate issues of gender diversity into student curricula and actively engage in discussions about sex, gender, and the limitations of skeletal methods with law enforcement and medicolegal professionals. It is also crucial that TGV individuals be included in these conversations.

**Embrace an advocacy role.** While some forensic scientists are personally uncomfortable with direct engagement in advocacy, embracing an advocacy role does not invalidate one's scientific objectivity. In fact, some cases may *require* advocacy to achieve resolution. Advocacy can include willingness to conduct case reviews with a gender-informed approach, revive attention on the case, seek resources outside of academia or the medicolegal system, and educate investigators about issues of sex and gender.

**Engage in and facilitate interdisciplinary collaboration.** The scope of fatal violence against TGV people is not yet understood, so the response to the epidemic must be multidimensional. Listening to, acknowledging, and uplifting the essential perspectives and expertise of non-academic experts, activists, and students (especially those who are TGV) aids in information exchange and leads to better research and casework strategies. The responsibility of meaningful allyship should fall on those in positions of power and privilege in the forensic sciences, including professors and conference organizers, to provide accessible opportunities and spaces for TGV researchers.

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### Gender Diversity, Transgender, Identification



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## A108 Disciplinary and Community Ethics Surrounding Gender- and Sex-Diverse Individuals in Forensic Casework

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**Learning Overview:** After attending this presentation, attendees will understand the roles of competency, responsibility, and non-maleficence in an ethical forensic anthropological practice with a particular focus on how to improve engagement with the Gender- and Sex-Diverse (GSD) community.

**Impact Statement:** This presentation will impact the forensic community by highlighting paths toward a more ethical practice within forensic anthropology that aims to improve outcomes for communities while also doing no harm.

The reconstruction of the biological profile as an extension of personhood and identity necessitates that forensic anthropology practitioners work within an ethical framework. The discipline of forensic anthropology lacks comprehensive ethical policies, largely due to the failure of relevant professional organizations to create, maintain, and enforce robust ethical codes.<sup>1</sup> Drawing on ethical principles outlined by neighboring fields (namely bioethics and the American Psychological Association), this presentation proposes key components of ethical practice in forensic anthropology, specifically in the context of research and casework involving GSD individuals. These components center on competency, responsibility, and non-maleficence as integral aspects of ethical practice.

Competencies are the various sets of skills needed to perform disciplinary tasks. There are several defined types of competencies, including those intended to meet changing social demands and minimize harmful downstream effects. For example, cultural, linguistic, and structural competencies are incorporated into medical curricula to understand and mitigate the complex interaction of social variables that affect medical care.<sup>2</sup> Forensic anthropology is uniquely positioned to provide a multidisciplinary perspective bolstered by the social sciences in defining and creating sets of competencies for forensic science. In the case of working with the GSD community, one such structural competency would involve engagement with social and structural factors that result in disproportionate violence against GSD individuals and a recognition of historical vulnerabilities within GSD populations. This engaged competency would promote ethical practice in a field tasked with identification of these victims.

As forensic anthropologists maintain an influential position within the medicolegal system, they have a responsibility to beneficent practice. Currently, methods to estimate biological sex do not acknowledge that sex is on a spectrum, nor do they provide space for a consideration of gender. While biological sex and gender are two separate concepts, they can be intertwined in an individual's identity and gender expression, which can affect the ability to identify a set of remains of an unknown decedent. While we work to establish protocols and better methods to incorporate the broader range of human variation and the human experience, we should ensure that our reports and scientific writing do not reinforce the concept of binary sex and gender roles.

Non-maleficence is related to beneficence but is more focused on preventing harm. There is currently a high level of interest but relative paucity in foundational research in both skeletal growth and development and forensic identification of GSD individuals. Increased considerations apply to the ethical use of GSD individuals as a source of data in research as they could represent a vulnerable population to the Institutional Review Board (IRB). Stakeholder participation in research design has been suggested as means of minimizing harm beyond the subjective risk assessment of the IRB and builds trust in a marginalized community that has historically been exploited in research.<sup>3,4</sup>

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**Ethics, Anthropology, Gender/Sex Diverse**

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## A109 Necropolitics and Trans Identities: Language Use as Structural Violence

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**Learning Overview:** After attending this presentation, attendees will better understand why inclusive language is an essential standard in the creation of representative and ethical systems of death investigation and deathcare.

**Impact Statement:** This presentation will impact those involved in death investigation by highlighting how lack of inclusive linguistics standards in case reports, death certificates, forensic methodology, human identification databases, and final disposition documents harms Transgender and Gender Non-Conforming (TGNC) individuals even after their death.

Despite the increasing visibility of TGNC people in United States society, current practices within medicolegal death investigation, forensic anthropology, and deathcare as a whole are not TGNC inclusive. This lack of consideration for TGNC decedents can cause unnecessary delays in the identification and disposition of their remains; moreover, it may ultimately lead to the non-consensual, forced postmortem detransition from their chosen identities. <sup>1</sup>

This presentation uses DeLeón's concept of necroviolence—"violence performed and produced through the specific treatment of corpses that is perceived to be offensive, sacrilegious, or inhuman by the perpetrator, the victim (and [their] cultural group), or both"—as a framework to illustrate how the (mis)use of language within death investigation and deathcare reflects and reinforces structural violence against TGNC people.<sup>2</sup> First, this presentation highlights the systemic failure to acknowledge differences between sex and gender. Examples from publicly available cases are then used to show how language and language-enforced bureaucratic structures can harm TGNC decedents, their surviving friends and chosen family, the broader TGNC community, and the process of medicolegal death resolution itself.

This presentation concludes by suggesting steps anthropologists, pathologists, death investigators, and their affiliated partners can take to reduce the systemic necropolitical violence faced by the TGNC community. While acknowledging that TGNC-inclusive methods will take time to develop and implement, it is essential that core standards appropriately serve these individuals. The core language used in death investigation will therefore need to disengage from binary and essentialist standards of "male/female" that fail to represent complex social and biological realities within human populations.

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- <sup>2.</sup> DeLeón, Jason. *The Land of Open Graves: Living and Dying on the Migrant Trail*. Oakland: University of California Press, 2015, 69.

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### Necroviolence, Necropolitics, Deathcare

## A110 Identifying Transgender and Gender-Variant Individuals in Public Records: Reconciling Differences Between Law Enforcement Data and Non-Government Organizations (NGOs)

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**Learning Overview:** After attending this presentation, attendees will understand where discrepancies may arise in calculations of Transgender and Gender Non-conforming (TGN) violence and homicide rates NGOs and Government Organizations (GOs).

**Impact Statement:** This presentation will impact the forensic science community by providing recommendations for determining TGN identity in database records and tracking rates of violent hate crimes and homicide involving TGN individuals.

Published reports on TGN violent hate crimes and homicide by GOs indicate that TGN groups experience comparable rates of violent hate crimes and homicide to non-TGN groups. However, published reports by LGBT+ NGOs indicate that TGN homicide is at epidemic proportions. This study builds on previous work examining these discrepancies to determine where these differences in calculations may arise and the impact of such differences.<sup>2,5</sup> An in-depth analysis of the Trans Doe Task Force's (TDTF) LGBT+ Accountability for Missing and Murdered Persons (LAMMP) database aids in this study of discrepancies by comparing self/personal reports with information gathered by the TDTF from the National Missing and Unidentified Persons System (NamUS).<sup>6</sup>

Counts of transgender homicides from 2013 through 2021 were collected from the published reports by the Federal Bureau of Investigation (FBI) Hate Crime Statistics Program, the Human Rights Campaign (HRC), and the National Coalition of Anti-Violence Program (NCAVP).<sup>1,3,7</sup> Slight differences were found between counts reported by the two NGOs and large differences were found between counts reported by the FBI and those reported by the NGOs. Of the three sources, HRC publications generally reported the largest number of homicides with steadily climbing numbers over time while FBI publications reported the lowest numbers with little-to-no change from year to year.

Differences in homicide counts arose from variation in the number and reporting methods of data sources. NGOs reported all TGN homicides documented by sources, including LGBTQ+ organizations and news reports. FBI data was sourced from participating law enforcement districts and restricted reported homicides only to those classified as a hate crime. The limited number of districts participating meant that FBI data did not span as much of the United States as NGO data did. Furthermore, it is unlikely that all participating districts recognized and documented TGN identities in reports or that all homicides involving TGN individuals were reported.<sup>4</sup>

Further analysis of the LAMMP database also highlighted discrepancies that NGOs deal with when working with GO data. Based on the LAMMP database of missing transgender persons reported, 77.6% of transgender individuals missing were trans women and 22.4% were trans men. However, when compared to NamUS data, cases that were flagged as possible transgender cases were overwhelmingly trans women, 91.4%, compared to trans men, 8.6%. This is a major discrepancy identified by directly comparing NGO data to GO data.

The results of this study demonstrate that NGOs and GOs calculate significantly different rates of crime against TGN individuals, even when using similar data sources due to their different methods of interpretation. Solutions to this discrepancy may come from incorporation of inclusivity practices, including the use of diverse gender categories on reporting forms, sensitivity training, and allying with TGN community liaisons to build better relationships with this at-risk population.

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### Transgender, Necroviolence, Anthropology

## A111 Identifying Trans Individuals From Skeletal Remains: Indicators of Gender-Affirming Interventions

*Angelica Bourgeault, MSc\*, University College London, London, Great Britain, United Kingdom*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the current state of research regarding the impact of Gender-Affirming Interventions (GAI) on the skeletal composition of transgender individuals and its implications for the forensic anthropologist.

**Impact Statement:** This presentation will impact the forensic science community by addressing the dearth in forensic literature on gender diversity, presenting evidence of the malleability of sexually dimorphic features that may be achieved through endocrinal and surgical GAI. Findings will guide experts through the identification process of skeletal remains and highlight the importance and potential of interdisciplinary collaboration.

A challenge is posed to the identification process when a person's skeletal features lead to conflicting or "indeterminate" sex estimations. Trans-identifying individuals may subject their bodies to an extreme degree of change by undergoing hormonal interventions and gender-confirmation surgeries to treat gender dysphoria and transition their physical appearance to align with their gender identity. Yet, despite disproportionate homicide rates of trans-identifying individuals, there are no forensic anthropology standards for determining whether an individual has undergone GAI from skeletal evaluation.<sup>1</sup>

This research aims to bridge the gap by proposing skeletal and medical indicators for the identification of trans individuals through a critical review of the current literature. The purpose of this project was four-fold to identify: (1) skeletal indicators for identifying trans individuals, (2) associated surgical devices, (3) differentiations between trans and cisgender (cis) surgeries, and lastly, (4) a proposed method for conducting the "sex estimation" of trans skeletal remains.

In terms of the skeletal impacts of endocrinal GAI, changes in cortical and trabecular areal and volumetric Bone Mineral Density (BMD) absolute values and z-scores/t-scores, bone mass, area, thickness, and turnover, as well as endosteal and periosteal circumference were analyzed. In addition, the effect of pubertal suppressors on epiphyseal fusion and final achieved height were explored, along with the effects of surgical implants on bone composition. Pubertal suppression and feminizing/masculinizing hormonal therapy, prior to and following gonadectomy, led to significant differences in bone density, morphology, and morphometry between trans individuals, specifically trans women, and their age-matched cis genotypic peers.

On the other hand, surgical indicators of GAI were determined by analyzing the effects of facial feminization/masculinization surgery and urological/gynecological gender-confirmation surgeries. The most reliable surgical indicators in trans women consist of combined evidence for bone shaving, various osteotomies, and breast implants. Conversely, the most durable postmortem surgical devices likely to lead to the positive identification of a trans man are the combined presence of facial implants and/or bone grafts, and penoscrotal prostheses.

Forensic anthropologists should be cautious not to confound evidence of surgical interventions with those used to treat traumatic, pathologic, or simple cosmetic procedures in the cisgender population. From a forensic anthropology point of view, GAI are most discernible and positively identifiable in individuals who have undergone the full extent of the transition and its hormonal and surgical effects on the skeleton. Nonetheless, findings may inform skeletal involvement in gender non-conforming, non-binary, or genderqueer populations undergoing lesser GAI, if any. In conclusion, the more the presence of extensive bilateral gender-affirming indicators, the higher the likelihood of ascribing the correct "sex" to an unidentified trans individual.

This research highlights the importance for forensics to remain up-to-date with innovative medical interventions of increasing popularity. The intricate relationship between sex and gender in trans individuals offers a unique opportunity to modernize the way forensic anthropologists interpret "indeterminate" scores. Future interdisciplinary collaboration and community involvement is key to better understanding the true extent of GAI impacts on the skeleton.

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### Forensic Anthropology, Sex Estimation, Transgender

## A112 Supporting the Positive Identification of Trans Men in Forensic Anthropological Contexts

*Jenna Schall, MSc\*, University of Toronto-Mississauga, Mississauga, Ontario, Canada; Tracy Rogers, PhD, University of Toronto-Mississauga, Mississauga, Ontario, Canada; Jordan Deschamps-Braly, MD, Deschamps-Braly Clinic of Plastic & Craniofacial Surgery, San Francisco, CA*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the rise in anti-trans violence and the role forensic anthropologists play in supporting the correct identification of trans individuals, particularly trans men.

**Impact Statement:** This presentation will impact the forensic science community by providing knowledge and additional tools to support and recognize trans men in forensic contexts.

The identification of unidentified human remains begins with the construction of the biological profile, which consists of an individual's sex, ancestry, age, and stature. This profile is then compared to descriptions of missing persons in order to narrow the scope of a forensic investigation. In cases of transgender and gender-diverse individuals whose sex assigned at birth has little correlation with their social identity, positive identification can be delayed or hindered.<sup>1-5</sup> There are many gender confirmation options for trans individuals who physically transition in order to alleviate gender dysphoria, such as hormone therapy, and genital and/or facial gender-confirmation surgeries.<sup>5,6</sup>

While not all trans people undergo these treatments for various reasons, undergoing gender-confirmation surgery should not hinder or complicate positive identification. Instead, forensic anthropologists need to be able to recognize evidence of gender-confirmation surgery in order to create a holistic and accurate biological profile, ultimately supporting correct positive identification. This growing body of knowledge is becoming increasingly more relevant and necessary as gender-affirming surgeries become more accessible for both trans women and men.<sup>5-9</sup>

Unfortunately, the number of trans and gender non-conforming individuals subjected to violence has also increased, due in part to the rise in anti-LGBTQ legislature being passed across the United States.<sup>10,11</sup> In 2020, the Human Rights Campaign (HRC) tracked a record number of violent fatal incidents against transgender and gender non-conforming people.<sup>11</sup> Forty-four fatalities were recorded by HRC, making 2020 the most violent year on record since HRC began documenting these crimes in 2013.<sup>11</sup> Also steadily increasing is the number of trans men and boys being reported as victims of these hate crimes.<sup>11,12</sup> While there is some preliminary research to support the identification of trans women in forensic anthropology, there is none on trans men.<sup>3-5, 13</sup>

The goal of this research is to illuminate the increase in reporting of trans men as victims of violence and review current gender-confirmation procedures to evaluate their potential in supporting the identification of trans men in forensic contexts.

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### Forensic Anthropology, Transgender Identification, Trans Men

## A113 When a Reanalysis Changes Everything: A Cold Case Study of a Possible Gender-Variant Individual

Alexis Goots, MA\*, Michigan State University, East Lansing, MI; Carolyn Isaac, PhD, Michigan State University, East Lansing, MI; Todd Fenton, PhD, Michigan State University, East Lansing, MI

**Learning Overview:** After attending this presentation, attendees will understand how cognitive bias can influence sex estimation in forensic anthropology.

**Impact Statement:** This presentation will impact the forensic science community by providing practitioners with an example of how to respectfully and scientifically address potentially gender-variant individuals in a cold case context.

The identification of an unknown decedent in forensic anthropology relies on the accurate estimation of the biological profile, including age, sex, stature, and ancestry. Although the methods for estimating the biological profile are well-established, many factors can impact whether these four components are estimated accurately. Intrinsic factors, such as ambiguous skeletal morphology, or extrinsic factors, such as personal effects, can lead to cognitive bias and increase the possibility of an inaccurately estimated biological profile. In these scenarios, the potential for a positive identification is limited.

When reanalyzing a cold case, therefore, an important first step is to determine whether the original analysis is accurate. Often, new methodology can improve accuracy in estimating the biological profile. Importantly, these re-analyses may reveal past mistakes, albeit made in earnest, that can help explain why a case has remained unresolved for a significant length of time. It is not only valuable to ameliorate these issues from a case resolution standpoint, but also to understand the circumstances that contribute to these inaccuracies, which may provide insight into broader issues in the field.

One such circumstance is the biasing effect of gendered clothing on sex estimation in forensic casework. A study of cognitive bias in sex estimation found that gender-stereotypical clothing associated with unambiguous skeletal remains had substantial biasing power, leading to inaccurate sex estimation despite the remains clearly expressing characteristics of a particular sex.<sup>1</sup> As gendered clothing can lead to inaccurate sex estimates and impact the ability to make identifications, its biasing effect should be considered when analyzing new cases and reevaluating cold cases in a forensic laboratory.

During a reanalysis of cold cases in the Michigan State University Forensic Anthropology Laboratory (MSUFAL), this study identified a case in which the reported sex was female, but the remains themselves appeared more ambiguous with respect to sex. The originally reported biological profile for this case was that of a Black female between the ages of 28 and 40 with a stature of approximately 65 inches. Notably, the decedent was found with personal effects, including a wavy, collar-length wig, blue jeans, a violet-colored long-sleeved blouse, a beige-colored long-sleeved blouse, a reddish-brown nylon neck scarf, panty hose, a bra, and black high-heeled shoes.

In the reanalysis, substantial disagreement between several anthropological methods resulted in an overall “indeterminate” estimation of sex. Ultimately, nuclear Short Tandem Repeat (STR) DNA analysis was used to identify the sex of this individual; the presence of X and Y chromosomes in the decedent’s DNA are consistent with a sex estimation of male. However, the stereotypically “feminine” personal effects in this case suggest that the decedent’s gender expression may have differed from their sex assigned at birth.

Given this information, the MSUFAL produced a follow-up report, noting the sex estimated using DNA analysis, as well as the potential for this individual to have been gender non-conforming in life. Although an identification has not yet been made in this case, the likelihood of eventually identifying this individual has been substantially improved based on the anthropological reanalysis. This case provides an example of the biasing power of extraneous information on skeletal analysis and the importance of thinking outside the gender binary in forensic casework.

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### Cognitive Bias, Sex Estimation, Gender Variance

## A114 A Diverse Past, Present, and Future: Resituating the Anthro in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will be introduced to the symposium “Pioneers of Color: A Diverse Narrative of Forensic Anthropology,” whereby the impetus, goals, and significance of the symposium will be outlined.

**Impact Statement:** This presentation will impact the forensic science community by supplying a richer understanding of forensic anthropology’s history than what is currently understood and provide space for discourses that disrupt the status quo.

For the first time at the American Academy of Forensic Sciences annual conference, a symposium is introduced that is entirely dedicated to the celebration of anti-traditional perspectives in forensic anthropology with a panel entirely composed of early-career scholars of color with diverse backgrounds. In providing spaces to disrupt the status quo, the contributors center the stories of early luminaries of color; the incorporation of feminist, critical race, and postcolonial theories in approaching medicolegal problems; and the dismantling of epistemological boundaries between science/activism and objectivity/subjectivity, among other pressing issues. In addition to introducing the symposium and presenters, the authors discuss why they are doing this, why it is essential, the difficulties they encountered in curating this content, and how these relate to the larger issues of representation and diversity within our field.

The content and presenters of this symposium align well with this year’s theme of a responsive academy, meeting and surpassing the challenges of a modern forensic science world. *Responding* to a modern forensic world begins by reclaiming forensic anthropology’s diverse historical foundations and acknowledging those routinely left out of the narrative. *Surpassing* the needs of a modern forensic world requires a counter-narrative to dominant renderings of forensic anthropology’s theories and praxis. This presentation and symposium also celebrates the Anthropology Section’s 50th Anniversary by bridging a biased past with a turbulent present and envisioning a more inclusive future for forensic anthropology and the forensic sciences.

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### Forensic Anthropology, Diversity and Inclusion, Biased Objectivity

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## A115 A Counter-Pantheon to Forensic Anthropology’s Classic Luminaries

*Jesse R. Goliath, PhD\**, Mississippi State University, Mississippi State, MS; *Matthew C. Go, PhD*, SNA International, Supporting the Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI; *Allison Nesbitt, PhD*, University of Missouri, Columbia, MO; *Aaron J. Young, MA*, University of Arizona, Tucson, AZ; *Sydney S. Garcia, MA*, SNA International, Supporting the Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI

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**Learning Overview:** After attending this presentation, attendees will better understand the important contributions of early non-White forensic anthropologists and related scientists and their contributions to the field.

**Impact Statement:** The goal of this presentation is to impact the forensic science community by highlighting the lack of diversity when teaching the history of forensic anthropology, its implications, and providing a solution to this gap.

The prevailing narrative of who has participated in forensic anthropology is arguably exclusive of non-White scholars and practitioners. Introductory textbooks in forensic anthropology and award names of the American Academy of Forensic Sciences Anthropology Section are examples of the types of people the field has traditionally regarded as worthy of exaltation. Our discipline retains a gaping hole in the historical record of important contributions led by those left out of the established pantheon. Unvaried participation in forensic anthropology creates a stagnant discipline that stifles innovation, impedes talent acquisition and retention by demoralizing the participation of non-White professionals, and obstructs the possibility to adapt to the needs of a just and equitable discipline. Through this presentation, there is hope to shed light on the lack of diversity among those widely cited as American forensic anthropology’s forebearers and to provide other luminaries to consider when teaching our history and considering names for section awards and accolades.

The format of this presentation adopts the widely used periodic system of forensic anthropology’s historical foundations, whereby its development is split between a Formative Period (Early 1800s–1938), Consolidation Period (1939–1971), and Modern Period (1972–Present). Within each period, this presentation introduces early pioneers such as W. Montague Cobb, Charles Warren, Caroline Bond Day, Tadao Furue, Kazuro Hanihara, Mahmoud El-Najjar, and others by highlighting their contributions to the discipline. Importantly, this presentation critiques the need to engage in this endeavor and why it has taken this long to do so and also reflects on the difficulties in curating this list and how it speaks to larger systemic issues of inclusion and recognition vis-à-vis race within the discipline of forensic anthropology.

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### History of Forensic Anthropology, Diversity, BIPOC



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## A116 Back to Black: The Legacy and Contributions of Black Pioneers of the 20<sup>th</sup> Century

*Isis Dwyer, MA\**, Department of Anthropology, University of Florida, Gainesville, FL; *Tisa N. Loewen, MA*, School of Human Evolution and Social Change, Arizona State University, Tempe, AZ; *Chaunese M.J. Clemmons, MA*, Department of Anthropology, University of Texas-San Antonio, San Antonio, TX and Department of Anthropology, Texas State University, San Marcos, TX; *Evonne Turner-Byfield, MSc*, Department of Anthropology, Ohio State University, Columbus, OH; *Jesse Goliath, PhD*, Department of Anthropology and Middle Eastern Cultures, Mississippi State University, Mississippi State, MS; *Allison Nesbitt, PhD*, Department of Pathology and Anatomical Sciences, School of Medicine, University of Missouri, Columbia, MO; *Carter Clinton, PhD*, Department of Anthropology, Department of Biology, Pennsylvania State University, State College, PA; *Kendra Isable, MA*, Department of Anthropology, University of Nevada, Reno, NV

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**Learning Overview:** Following this presentation, attendees will know the impactful contributions of 20<sup>th</sup>-century Black anthropologists who pioneered our field and will have a tangible resource of media that can be used as tools for education and citation.

**Impact Statement:** This presentation will impact the forensic community by highlighting the research of Black scholars and practitioners to the field of forensic anthropology and illuminating their continuous contributions.

Black biological anthropologists have made significant contributions to forensic anthropology and continue to contribute to the scientific field. Yet these theoretical, educational, scholarship, and technical contributions have been overlooked in undergraduate and graduate degree courses, forensic anthropology textbooks, and the forensic literature. The research of Black pioneers in forensic anthropology is not broadly shared, and inclusion of the work, ideas, and hypotheses of these anthropologists would greatly enhance the state of the discipline.

Forensic anthropology draws upon multiple medicolegal and social disciplines and is most effective at applying methods when consideration of the socio-cultural consequences are taken. Like their contemporaries, early Black anthropologists whose work impacted forensic anthropology were educators and researchers with diverse research interests and careers. They were biological anthropologists, cultural anthropologists, and applied anthropologists who worked with the military, anatomists, and forensic psychologists.

William Montague Cobb, the first African American to complete a doctorate in biological anthropology, employed multidisciplinary methods across physical anthropology, anatomy, and human biology to refute the perceived differences between Black and White Americans, counter the scientific justifications for racism, and explore health disparities. Cobb's work exemplifies a pioneering instance of applied anthropology and social responsibility that provides vital context to current constructions of population variation utilized in forensic ancestry estimation today.

Charles Warren was a contemporary of Furue and Kerley and worked occasionally for 25 years as a civilian forensic anthropologist for the United States Army and identified human remains from World War II, the Korean War, and Vietnam War. As a professor and educator, he consulted on forensic cases in Illinois and Indiana. His most high-profile case was identifying human remains from the house of John Wayne Gacy in 1975. As a researcher, he dispelled the belief that because of their skin color, the Black Asian population in the Philippines were a homogenous group.

Key figures in forensic psychology, Drs. Mamie and Kenneth Clarks' "doll test" and research were instrumental in demonstrating the harmful effects of segregation in *Brown v. Board of Education of Topeka* (1954). Likewise, Caroline Bond Day, a student of Earnest Hooton at Harvard, used genealogies, interviews, and anthropometrics in her expansive 1932 Master's thesis to show that laws prohibiting miscegenation predicated on biological inferiority were baseless.

When exploring the contributions of Black pioneers of the 20<sup>th</sup>-century, it is difficult to quantify their impact in parallel to their non-Black peers. Many early Black scholars in the forensic disciplines faced considerable obstacles to traditional markers of academic success, such as publications and tenured promotion, while others were pushed out of the discipline altogether. Despite these obstacles, the legacies of Black pioneers in forensic anthropology live on not only through distinguished labs and collections, but also their influence on the theory and praxis of current Black scholars and practitioners. While current events have revealed our discipline's intent to maintain "objectivity," Black pioneering scholars maintained passionate rigor in activist scholarship while emphasizing the importance our discipline has on the everyday world. A clearer understanding of the scholarship of Black anthropologists is alive and offers critical perspectives and diversity necessary to enhance our discipline.

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### History, Diversity, Forensic Anthropology

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## A117 Drawing Parallels: Black, Indigenous, and People Of Color (BIPOC) Representation in Other Medicolegal Fields

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the scopes of BIPOC representation in different medicolegal fields, as well as the strategies or initiatives to increase BIPOC representation proposed by different medicolegal fields.

**Impact Statement:** This presentation will impact the forensic community by providing data on diversity, inclusion, and representation from different medicolegal fields and by identifying the strategies used or proposed to achieve goals in supporting diversity, equity, and inclusion.

The benefits of a diverse and inclusive working environment have been well-documented. This is especially needed in forensic sciences, where new scientific advances can greatly benefit society. Underrepresentation may lead to a bias that ideas and topics that are culturally and/or biologically significant to certain groups are less important to the community as a whole. Additionally, there is a general mistrust and negative connotations associated with law enforcement and the medicolegal system among people of color.

This study surveyed the literature and data reported from professional organizations in an attempt to find information about diversity within different subdisciplines of medicolegal fields. Results showed that for anthropology, 7% of members in the American Association of Biological Anthropology (AABA) identified as being an underrepresented minority, while about 32% of members of the Anthropology Section of the American Academy of Forensic Sciences (AAFS) self-identified as being a member of an underrepresented group. However, the Anthropology Section represents less than 4% of the total membership of the Academy. There is no direct data on BIPOC representation in other medicolegal fields. However, insights can be gleaned from statistics of the greater scientific communities. In pathology, about 15% of the trainees (pathology residents) in 2016 reported to be members of an underrepresented minority. Significant differences were also found in terms of underrepresented minority pathology fellows and residents compared to the United States population. The Society of Toxicology (SOT) also openly acknowledged there is racial, ethnic, and gender inequality in participation and leadership within the society, but did not report actual statistics regarding their membership or leadership compositions. This study acknowledges that its results may not represent accurate BIPOC representation in medicolegal fields, but they all signify an overall problem of a lack of diversity. Acknowledgment and recognition is not enough to mitigate the trend of underrepresentation. It is imperative that we implement, in earnest, strategies to diversify the scientific community as a whole. While not exhaustive, some of these strategies may include: acknowledgement of differing experiences for BIPOC individuals; early education outreach; encouragement and access to opportunities throughout educational and career trajectories; and correcting compensation inequalities.

Finally, this study recognizes that while different statistics from different organizations or research projects may not be directly comparable (as the definition of “underrepresented group” may differ), professional organizations are encouraged to be open about the diversity (or lack thereof) in their membership. This openness is beneficial in identifying strategies to improve BIPOC representation within their fields. Without this transparency, we, as a scientific community, run the risk of a narrowly focused approach to solutions and services that affect the scientific community as a whole.

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### Diversity, Representation, Medicolegal

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## A118 Objectivity in the Historical Narrative of Forensic Anthropology

*Kamar Afra, MA\**, University of Tennessee, Knoxville, TN; *Elaine Y. Chu, MA*, University of Nevada, Reno, Reno, NV; *Sydney S. Garcia, MA, SNA International, Joint Base Pearl Harbor-Hickam, HI*

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**Overview:** After attending this presentation, attendees will have gained a more in-depth understanding of the historical factors that led to enhanced racial categorization in science and anthropology and how this translated to the othering and exclusion of Black, Indigenous, and People Of Color (BIPOC) professionals.

**Impact Statement:** The goal of this presentation is to understand some of the tools that impacted the perspective of historical and current anthropologists and impede the contribution of BIPOC experts.

The objectivity of forensic science has been tangled in quantitative analysis due to expectations outlined in legal proceedings. Numerous fields rely on statistical tests to uphold the authenticity and admissibility of a result.<sup>1</sup> Statistical analyses and classification accuracy rely on that—the assumption that groups are distinct (based in scientific truth). When applied to humans, these groupings are grounded in oppressive socio-political hierarchies that are embedded in subjective thought.

In the late 19th and early 20th century, the predisposed ideas of human typology and xenophobia popularized anthropology. The eugenics movement reiterated the concept of “culture as biology” by linking genetic determinism, savagery, and racial classification.<sup>2</sup> This movement fueled the research of early anthropologists and other scientists who were working in a forensic sphere.

Historically, only those in power who created and benefited from the hierarchy were heard. Thus, objectivity of experts was defined by this subjective bias. Expert bias has been the seed for several fields, such as anthropological studies, that linked cranial capacity to intellect and levels of humanity to cranial morphology. These contributions to the field promoted and validated the othering of prominent people of color professionally and socially, such as W.E.B. Du Bois, Caroline Bond Day, and Anténor Firmin.<sup>3</sup> This presentation provides a snapshot of the interdependence of conscious “objectivity” with an active effort to dismantle the theory of racial inferiority driven by eugenics ideas that contributed to the masking of BIPOC experts’ contributions for more than a century. Human variation does not equal racial or ethnic categorization, and understanding the history that led to these assumptions is necessary to decrease unconscious expert bias and defer scientific racism. Forensic anthropology should be anthropology first.

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### Forensic Anthropology, Expert Bias, Objectivity

## A119 Forensic Anthropology's Role in Activism and Advocacy

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**Learning Overview:** After attending this presentation, attendees will understand the need for activism to be ever-present in the work of forensic anthropologists. Hearing from minorities in the field will ensure that attendees will be presented with a variety of points of view that are often unknown or have gone unnoticed.

**Impact Statement:** This presentation will bring about a discussion that is much needed in today's current climate regarding activism, human rights, and the role/effect of racism in forensic anthropology. This presentation was created and is presented by early-career persons of color in the field to shed light on the points of view of these individuals and communicate them to the community at large.

There is no space for objectivity in forensic anthropology because impartiality favors the side of the oppressor. This places an obligation on the forensic anthropologist to become an activist with their work and daily lives. Anything from non-profit work to inclusive teaching practices are considered acts of advocacy forensic anthropologists can pursue to fulfill that obligation. For Black, Indigenous, and People of Color (BIPOC) in this field, the act of existing in White academia and professional spaces is activism.<sup>1</sup>

Historically, activism has changed the field and evolved the practices of forensic anthropology into becoming more humane. It is undeniable that the work of activists has changed the field for the better. This has relied heavily on BIPOC activists like W.E.B. Du Bois and St. Clare Drake and their work.<sup>2</sup>

Forensic anthropologists are often hired to assist with the exhumation and identification of individuals who have died tragically from genocide and other violations of human rights. Forensic anthropologists aiding in this type of investigation are able to use their authority to advocate and bring awareness to the injustices that caused the demise of innocent people. Oftentimes being a part of this type of work also means working with *living* communities who are continually affected by the same injustices in their day-to-day lives. Forensic anthropologists can then advocate helping alleviate turmoil and support affected communities.

The belief that advocacy and activism do not have a place in forensic science comes from a place of privilege that BIPOC scientists do not have. These should not be mutually exclusive, especially when the public relies on the advocacy of scientists to improve the system by recognizing its flaws and limitations. There should be an incorporation of activist research.<sup>3</sup> This is further supported by organizations and individuals such as Carlina de la Cova, Michael Blakey, the Argentine Forensic Anthropology Team (EAAF), The Peruvian Team of Forensic Anthropology (EPAF), and the Forensic Anthropology Foundation of Guatemala (FAFG) that have and continue to use advocacy and activism as a tool to better support underserved communities.<sup>4</sup>

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### Anthropology, Activism, Human Rights

## A120 The (Colonial) Impacts of (Western) Forensic Anthropology in the International Sphere

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**Learning Overview:** After attending this presentation, attendees will understand and be able to recognize past and current practices of Western forensic anthropology that are deemed normal but are colonial in nature.

**Impact Statement:** The goal of this presentation is to impact the forensic science community by identifying issues with parachute forensic anthropology and suggesting a set of working guidelines to decolonize current practices in international collaborations in forensic anthropology.

Recent work has shown that Western nations, especially the United States, lead research in forensic anthropology significantly more than non-Western counterparts by absolute count of publications and citation impact.<sup>1</sup> This finding supports sentiments that the research and practice of forensic anthropology within Western contexts dictates the discipline's formation and exports their ideas to other parts of the world. This presentation seeks to highlight the inverse, where Western practice can stand to learn from non-Western contexts in their approaches. International collaboration in forensic anthropology takes place in both academic and professional contexts. Current practices in international forensic anthropological fieldwork typically involve the Western anthropologist traveling to a given host country to collect data at institutions or participate in excavations and promptly departing once the job is completed, a *modus operandi* known as parachute science or helicopter research.<sup>2,3</sup>

One example can be found in forensic humanitarian work, a well-established subfield of forensic anthropology that has been discussed extensively in the literature. Such work, often taking place in countries that are not part of the global West, is seldom spearheaded by local specialists. However, forensic humanitarian efforts often require context-specific approaches to be successful. Past experience also highlights the value of capacity-building and training in the sustainability of humanitarian projects.

To date, literature on ethics and professionalism in forensic anthropology does not provide explicit guidelines for working in foreign nations. Further, differences in how institutions across the globe operate call for the Western anthropologist to adapt to appropriate sensitivities when working in an international sphere. As such, protocols for how international collaborations should be conducted in forensic anthropology are relatively loose. Given a racist and colonial past in the history of Western anthropology, especially when dealing with international research, this study contends that guidelines for conducting international research should be specified.

This presentation aims to provide actionable steps by: (1) proposing working guidelines for international collaboration; (2) encouraging foreign experts to involve the local community in projects on equitable footing; and (3) ensuring data sharing and accessibility to additional materials. Through these steps, this presentation aims to provide a space for the local communities in various host countries to advocate for themselves and promote the decolonization of current practices within forensic anthropology.

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### Parachute Anthropology, Research Ethics, Decolonization

## A121 Homework for Modern Forensic Anthropologists

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**Learning Overview:** After attending this presentation, attendees will understand the barriers currently preventing retention of diversity in forensic science and how each attendee may work toward a more diverse and inclusive discipline.

**Impact Statement:** The goal of this presentation is to impact the forensic science community by providing actionable items to bring to their own institutions to foster a more inclusive work environment and accommodate the diversity of a modern forensic world.

The Anthropology Section at the American Academy of Forensic Sciences (AAFS) has seen a sharp increase in diversity of attendance and membership since the inclusion of undergraduate students as Student Members and the creation of the Diversity and Inclusion Committee. Fifteen years ago, a symposium featuring only early-career researchers who identify under the umbrella-term of Black, Indigenous, and People of Color would not have been possible, if not due to fear of reprisal, then simply due to a lack of numbers. While this symposium has highlighted the strides in ethnic diversity seen by AAFS membership and the advancements in thoughts and perspectives that come along with it, it is important to highlight the intersectionality of identity and acknowledge the other improvements to diversity that the section has achieved.

Research shows that increasing diversity is only the first step in creating a more collegial scientific environment—the “leaky pipeline” persists as a barrier to true diversity at the highest levels of academia and industry.<sup>1</sup> Diversity has often been used by institutions as a commodity, simply a numbers game in which a certain goal is determined by the dominant group and once achieved, is checked off as complete.<sup>2</sup> The general justification for increasing diversity is that diversity improves science by bringing new perspectives to foster innovation.<sup>2,3</sup> Such expectations then unduly rely on underrepresented groups to perform at levels higher than that expected of their majority peers.<sup>3</sup> Yet novel concepts from underrepresented groups are less likely to be recognized and/or adopted by their disciplines.<sup>4</sup> Therefore, it is important to intentionally dedicate space and time toward recognizing and celebrating all forms of diverse perspectives—especially in forensic science.

Drawing from diversity and inclusion research, this presentation evaluates the current state of diversity and inclusion efforts in the Anthropology Section of AAFS. Further, recommendations are provided for increasing *inclusion* in the discipline for higher *retention* of diversity.

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### Diversity, Inclusion, Retention

## A122 Quantification of the Mincer et al. (1993) Age Estimation Method Using Radiographs

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**Learning Overview:** At this presentation, attendees will be introduced to a method developed with the goal of quantifying the subjective, stage-based Mincer et al. age estimation method.<sup>1</sup> Specifically, attendees will become familiar with the advantages and limitations of using sectioning points derived from odontometric measurements from radiographs to assign developmental stages of the third molar.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the high reliability, but variable accuracy, of assigning developmental stages of the third molar based on odontometric measurements from radiographs. Furthermore, this study will attempt to validate the Mincer method on a sample of, mostly young, adult males that includes individuals with impacted molars.

Experience plays an important role in the age estimation of human remains, particularly for methods that are based on scoring subjective stages. This can leave inexperienced observers at a disadvantage. Given that research has shown metric measurements are typically reliable even when measured by observers with varying experience levels, stages assigned based on metric measurements may yield estimates of age that are more accurate and reliable, especially by less-experienced observers.<sup>2</sup> This study developed an age estimation method assigning developmental stages of the third molar based on sectioning points derived from metric measurements, and subsequently assessed the reliability and accuracy of the method. Additionally, the validity of the original Mincer method was also evaluated on a sample of adult males who were mostly young adults.

This study used radiographs of third molars from 49 casualties of the World War II USS *Oklahoma* incident housed by the Defense POW/MIA Accounting Agency Laboratory, which was comprised of 17–52-year-old males. For each individual, five measurements were taken from a radiograph of at least one mandibular third molar and measurement ratios were calculated. Measurement ratios that significantly correlated to each Mincer stage of development were used to establish sectioning points that differentiated the developmental stages. To estimate the reliability of the stages assigned based on sectioning points, a separate sample of 12 third molars were re-measured and re-scored after one week. To assess the accuracy of the stages assigned based on sectioning points for each of the measurement ratios, stages from the 12 third molars were compared to stages previously assigned by experienced forensic anthropologists using the original method. Finally, to validate the original Mincer method on this sample of young adult males, the estimated age using the original Mincer method was compared to the known age of the individuals.

The results indicate excellent intra-observer reliability for obtaining the stages from the sectioning points, with Intra-Class Correlation Coefficient (ICC) values of at least 0.972. However, the accuracy of assigning stages was variable, with correct classification ranging from 25% to 75% for the different measurement ratios; the apical width ratios were the most accurate. Finally, application of the Mincer method on this sample provided age estimates that, on average, were younger than the actual age for all of the stages. However, the sample included individuals with impacted molars, which recent research has shown can result in underestimation of age.<sup>3</sup>

According to this study, sectioning points derived from metric measurement ratios of the third molar using radiographs are reliable (i.e., successfully replicated); however, the use of these sectioning points to accurately assign Mincer stages of development for age estimation is not supported due to low accuracy, with the exception of the apical width measurement ratios. Additionally, the findings support previous research that the use of third molar development for age estimation generally provides underestimates of age, which may in part be due to the inclusion of impacted teeth.

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### Age Estimation, Third Molar, Sectioning Points

## A123 Does Structural Violence Impact Forensic Anthropological Age Estimation? Investigating Skeletal Indicators of Biological “Weathering” in Modern United States Individuals

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**Learning Overview:** After attending this presentation, attendees will be familiar with the weathering hypothesis—the concept that Black Americans’ health begins to cumulatively decline in early adulthood, with embodiment as its mechanism—and its potential skeletal impacts.

**Impact Statement:** This presentation will impact the forensic science community by showcasing how the lived experiences of social inequity and structural violence can become embodied, affecting individuals on the skeletal level and in turn affecting the accuracy of forensic anthropological age estimations.

Accuracy in estimating the biological profile is imperative to the medicolegal personal identification process. Yet, most forensic anthropological methods have not been generated or tested on the remains of people of color—the very populations global forensic anthropological casework most frequently serves. The donated skeletal collections on which many biological profile methods are based do not reflect global population demographics, containing disproportionately high numbers of individuals who identified as “White.”<sup>1</sup> Methods developed on the remains of relatively privileged United States White individuals may not be appropriate in many domestic medicolegal and international human rights contexts.

The disconnect between method production and the reality of medicolegal casework is highlighted by decades of public health research, such as the weathering hypothesis.<sup>2</sup> The weathering hypothesis states that the overall health of Black Americans declines cumulatively, beginning in early adulthood, compared to White Americans, due to living with the chronic stress of social inequity. Members of different cultural, religious, socioeconomic, and racial groups experience and embody different stressors over the life course, with the social becoming biological in a process that may be discernible skeletally.<sup>3</sup> Embodiment and weathering have direct relevance to the biological profile—specifically, age estimation—as the remains of prematurely weathered individuals may “look older” than their chronological age and thus result in an inaccurate age estimate. However, the potential for skeletal weathering has yet to be investigated.

In order to test whether premature aging (i.e., weathering) is discernible in human skeletal remains, 183 skeletal individuals from the W.M. Bass Collection were analyzed for several age indicators, “blind” to their demographic data. Each individual was scored on: Antemortem Tooth Loss (AMTL) via the Eichner index; the Suchey-Brooks pubic symphysis aging method; and the San-Millán-Rissech acetabulum aging method.<sup>4-6</sup> The sample included White ( $n=127$ ) and non-White ( $n=56$ ) self- or kin-donated individuals. The White sample included lower-Socioeconomic Status (SES) males and females ( $n=63$ ) and higher-SES males and females ( $n=64$ ), and the non-White sample included Black ( $n=31$ ), Hispanic ( $n=9$ ), and “Biracial” ( $n=16$ ) individuals regardless of SES. It was hypothesized that the non-White individuals would “score older” on all age indicators compared with both White sub-populations, and that lower-SES White individuals would “score older” on all age indicators compared with the higher-SES White sample.

The hypothesis that non-White individuals would “score older” was supported in one acetabular rim variable (V3; Kruskal-Wallis run in  $R$ ;  $p<0.05$ ). When only high-SES White individuals were included, however, non-White scores were also significantly higher for Suchey-Brooks phase and the acetabular fossa variables V6 and V7 (Kruskal-Wallis;  $p<0.05$ ). The hypothesis that lower-SES White individuals would “score older” than high-SES was supported for AMTL (Kruskal-Wallis;  $p<0.001$ ) and one acetabular fossa variable (V6; Kruskal-Wallis;  $p<0.05$ ).

These preliminary results highlight that skeletal aging is neither monolithic nor simply biologically determined. It is, to some degree, influenced by individuals’ lived, embodied experiences—potentially including both race- and class-based inequities. This evidence encourages the development of aging methods on large and diverse samples and highlights the role of biocultural processes in patterning human skeletal variation.

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### Age Estimation, Weathering, Embodiment



## A124 The Combined Use of Craniometrics and Odontometrics to Estimate Parameters of the Biological Profile

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**Learning Overview:** After attending this presentation, attendees will better understand how measurements of the cranium, mandible, and the dentition are correlated and how they may be used together to estimate parameters of the biological profile.

**Impact Statement:** This study will impact the forensic community by identifying how multiple data sets can be used together to arrive at improved estimates of the biological profile.

In methods to estimate parameters of the biological profile, practitioners often employ only a single variable type. For example, metrics and non-metric traits are not typically combined, nor are different types of metric variables (e.g., cranial metrics are analyzed separately from postcranial metrics). There are some exceptions to this finding, however. For example, Berg and Kenyhercz employ metric and non-metric variables to estimate population affinity and sex on mandibles, and there has been work to explore the relationship of cranial macromorphoscopies and dental morphology to understand population variation.<sup>1,2</sup> However, there has not been work exploring the relationship of cranial metrics and dental metrics and how the incorporation of both data sets may improve estimates of the biological profile. The goals of this project are to identify how measurements of the cranium and mandible are correlated with those of the dentition and to use this information to combine datasets in a biologically meaningful way to test if this dataset can improve estimates of the biological profile.

Data were collected from the Texas State University Donated Skeletal Collection. This study collected data on cranial and mandibular metrics, then dental metric data on the same individuals ( $n=57$ ) were collected. Correlations among the variables were noted to identify correlated patterns of growth and development of the craniofacial complex. A network analysis was conducted to identify modules of interrelated variables. The largest module was then used in a Discriminant Function Analysis (DFA) to explore the ability of the variables in the module to estimate biological sex and population affinity as identified via self-reported social race and sex categories. The results of this DFA were compared to a stepwise DFA to identify which model performed better. All statistical analyses were done in the R statistical computing environment and SPSS 26.<sup>3,4</sup>

Pearson Correlation coefficients show that the vast majority of variables were positively correlated. Many of the measurements of the face were highly correlated with measurements of the teeth, predominantly measurements of the lower third molar. These results identify correlating patterns of growth and development between the face and the dentition. The stepwise DFA for population affinity estimates identified four metric variables of the teeth and was accurate in 55.2% of cross-validated cases. The network analysis identified a module of 12 craniometric and 15 odontometric variables that contained the highest percentage of variables within the network. These variables (with the exception of the third molar measurements) were then used in another discriminant analysis. This revised model was able to correctly classify individuals in 86.2% of cross-validated cases, significantly improving on the original step-wise model. The same process was undertaken to estimate biological sex. The stepwise DFA identified four craniometric and five dental metric variables and was correct in 93% of cross-validated cases. In this case, the DFA using the variables from the network analysis module was less accurate (68.4% accuracy in cross-validated cases).

This work highlights the complexity of skeletal variation and how it may or may not correlate to a binary of biological sex and self-reported social race categories. However, generally incorporating measurements of the cranium, mandible, and dentition served to provide a more robust picture of human variation. It is important when studying modern human variation to carefully consider the phenotypic traits employed in analyses and to understand their evolutionary background. Future research would further investigate the impact of variable choice on statistical estimates and explore the potential negative impact of reinforcing biological categories of sex and population affinity within forensic anthropological research and casework.

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### Dental Metrics, Mandible, Cranium

## A125 Validating the Use of Osteoarthritis as an Indicator of Age in Human Skeletal Remains

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the Utility of Osteoarthritis (OA) in estimating the age of human skeletal remains.

**Impact Statement:** This presentation will impact the forensic science community by illustrating how OA—previously proposed as an age indicator for United States individuals who identified as White—was independently validated and its application expanded to include a more diverse sample.

Age at death is a critical aspect of the biological profile that forensic anthropologists generate from a set of human skeletal remains. However, current methods of age estimation rely on select bones, such as the innominates or fourth ribs, and if these bones are missing or damaged due to dismemberment, animal scavenging, or other taphonomic processes, then their utility for age estimation is limited. One way to remedy this methodological shortcoming is to investigate regions of the body that could be age-informative even in fragmentary contexts. Age estimation using OA is an example of one such approach.

In 2019, Winburn and Stock demonstrated that despite the multifactorial nature of OA, age was the only variable proven to predict the presence or absence of OA within a joint.<sup>1</sup> The presence or absence of OA in a given joint surface is therefore able to provide an estimation of age. The current study independently validated the results of Winburn and Stock on a sample of United States individuals.<sup>1</sup>

Using a binary scoring system, every appendicular joint surface (and the Temporomandibular Joint [TMJ]) from 275 self- or kin-donated skeletal donors from the William M. Bass collection was evaluated for presence or absence of OA ( $n=12$  American Indian or White/American Indian,  $n=4$  Asian,  $n=31$  Black,  $n=9$  Hispanic,  $n=223$  White). Using a binomial Generalized Linear Model (GLM) probit link function analysis, transition analysis enabled the prediction of the age at which an individual would transition from “no OA present” to “OA present” in a given joint surface.<sup>2-4</sup>

All statistical analyses were performed in *R Studio* (version: 1.4.1717; *R Core Team*, 2021). Alpha levels were set at  $p=0.05$  for all analyses. The “VGAM” package in *R Studio* enabled the probit link function of binomial GLMs to use log-scaled age to generate the modal age-at-transition, standard deviation, and estimates of the ages at which 90 and 95 percent of the population will have transitioned, for males and females.<sup>5</sup> In males, estimates of the age at transition (of “no OA present” to “OA present”) for 90% of the population ranged from 32.9 (knee) to 50.8 years (foot), and for 95% of the population from 37.5 (hip) to 87.4 years (TMJ). The age at transition to “OA present” estimate for 90% of the female population ranged from 28.0 (elbow and hip) to 48.7 years (foot) and for 95% of the population from 29.9 (elbow and hip) to 61.3 years (TMJ). When the two sexes were pooled, the age at transition estimate to “OA present” for 90% of the population ranged from 31.2 (knee) to 49.7 (foot) and from 35.4 (hip) to 73.5 (TMJ) for 95% of the population. In general, ages at transition were consistent with the findings of Winburn and Stock. Further, this study confirmed the lack of correlation initially reported by Winburn and Stock between OA and age in the female ankle joint, in addition to identifying that the male elbow is a problematic joint for OA-based age estimation. These joints should not be used to estimate age in these groups.

Since OA has been demonstrated to have a significant and reliable relationship with age, it has the potential to serve as a valuable tool for forensic anthropologists in their efforts to age human skeletons—particularly in fragmentary contexts.

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### Forensic Anthropology, Osteoarthritis, Age Estimation

## A126 An Evaluation of Palate Shape Scoring Systems

Christopher Maier, PhD\*, Eckerd College, St. Petersburg, FL

**Learning Overview:** After attending this presentation, attendees will understand differences in application and performance of existing systems for evaluating palate shape as a macromorphoscopic cranial trait for assessing population affinity.

**Impact Statement:** This presentation will impact the forensic science community by directly comparing and evaluating several existing systems for recording palate shape. This will allow practitioners who estimate population affinity to be informed regarding the replicability and efficacy of the available methods.

Palate shape has a long history as a cranial macromorphoscopic trait, appearing even in some early attempts to describe population-based human variation.<sup>1</sup> Consequently, over the decades, many different systems for recording palate shape have been developed.<sup>1-6</sup> However, with so many methods available, choosing the most appropriate method can be difficult, especially when direct comparison is unavailable. This presentation examines a selection of methods and evaluates their replicability and the strength of their association with population affinity to establish the pros and cons of each.

A sample of 670 individuals was evaluated from digital photographs taken of the cranium from an inferior view. Each palate was given a score according to the guidelines set forth by each of the palate scoring methods under consideration.<sup>1-6</sup> Method replicability was measured by the rate of observer error as measured by Cohen's weighted Kappa statistic.<sup>7</sup> Association with population affinity was measured with a variety of association statistics, including chi-squared tests, Cramér's V, Sakoda's corrected contingency coefficient, and correspondence analysis.<sup>8</sup> Finally, to assess overall performance, each method was ranked based on the statistics generated by the previous analyses. The sum of the ranks for each method represents an overall score relative to the other methods examined.

With respect to replicability, all the methods were recorded with substantial agreement or better ( $K > 0.72$ ) following the guidelines of Landis and Koch, though one part of the component system (anterior curvature) was less consistent with only moderate agreement ( $K = 0.578$ ).<sup>9</sup> Although all the systems are robust to observer error, methods with fewer options, like the three-shape system of Gill, are more replicable ( $K = 0.83$ ) than other systems.

As concerns association with population affinity, every system exhibits statistically significant differences, which is consistent with a relationship between palate shape and affinity. However, specific measures of association between scores and population affinity reveal a more complicated relationship. The effect size of the differences detected by the chi-squared tests, as measured by Cramér's V, is weak overall ( $0.11 < V < 0.17$ ).<sup>10</sup> Although the association is weak, the strongest association observed is that of the Gill method; however, one component of the component system, angle of the sides, did have a stronger association.<sup>2,4</sup> In contrast, a different pattern emerges if association between method scores and population affinity is measured with a contingency coefficient. Using  $C$  as measure of association, the scores in the component system and the 5-shape system proposed by Maier are the most strongly associated.<sup>4,5</sup> Finally, when considering both replicability and association together, the Gill and Maier shape systems appear to be the best systems.<sup>2,5</sup> Noteworthy is that the angle component alone performed as well as the Gill system.<sup>2,4</sup>

In conclusion, simpler systems produce results that are easiest to replicate, while systems with more levels and the component system bear a stronger association with population affinity.<sup>2,4,5</sup> The strong performance of the component system, and one of its components alone, suggest that a component approach may be viable going forward. Each of the methods examined offers advantages and disadvantages depending on if the practitioner more strongly weights replicability, association with population affinity, or a balance of the two.

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### Forensic Anthropology, Palate Shape, Methods Comparison

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## A127 A Pilot Study Re-Examining the Hartnett Adult Age-at-Death Estimation Methods as Component Systems

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the contributions of individual features within the Hartnett methods for estimating the age of individuals over the age of 45 years and the potential benefits of reconfiguring these phase-based methods into a component system.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the potential for a revision of the Hartnett age-at-death estimation methods from phases into components.

The Hartnett phase-based methods for the pubic bone and fourth rib were published in 2010.<sup>1</sup> They were derived largely from features described in historically popular methods, but provided updated trait descriptions, included several new features potentially indicative of advanced age, and provided age intervals based on large, contemporary reference samples. For these reasons, these updated methods have largely replaced earlier approaches for those regions of the skeleton in forensic practice.

The Hartnett Methods, like all phase-based methods use a series of ordinal categories, each containing descriptions of multiple characteristics that typify an anatomical region, which are associated with an age interval calculated from a reference sample. Because such methods include multiple features within each phase description and it is not uncommon for an individual to possess features of multiple phases, a description of an individual's morphology cannot be inferred solely from a phase designation (e.g., Phase 5). This prevents the evaluation of the contributions of individual features to age estimates.

Therefore, this pilot project reconsidered the Hartnett methods as component systems. A component scoring system was developed by identifying individual features present in multiple Hartnett phase descriptions and generating character states based on changes in each feature between phases. Collectively, 5 traits were identified for the fourth rib, and 11 traits were identified for the pubic bone, with between 3 and 7 character states for each.

All traits were scored from 77 individuals (males:  $n=41$ , females,  $n=36$ ) with documented age-at-death from the John A. Williams human skeletal collection at Western Carolina University. This collection primarily represents older White females and males from North Carolina and the surrounding states with most individuals (72/77) being over 45 years of age (average age: 64.9 years). All traits were scored on both the right and left sides for each individual. Because of these sample limitations (e.g., size, age distribution), this pilot project focused on evaluating the traits individually to assess their contributions to age estimates for older individuals, which could lead to the development of improved methods.

For all pubic symphysis components, essentially all individuals over the age of 45, were scored as the oldest two or three character states, depending on the trait. Weak age-related patterns were found for porosity and bone quality variables. Similar patterns were found for rib components, with weak age-related patterns identified for bone quality and the development of bony projections. The substantial overlap among the age distribution of individuals scored within each category indicates that the majority of these variables contribute little-to-no information to age estimates beyond the age of 45 years.

These data indicate that conversion of the Hartnett phases into component systems is unlikely to dramatically improve age estimates for older individuals. However, the use of a component system would improve documentation of the features used to estimate age, as well as providing the type of data necessary for research investigating variation in the aging process. As the pubic symphysis and sternal ribs are commonly used areas of the skeleton for adult age estimation around the world, the development of a component-based method using a large, modern sample and incorporating features of advanced age is recommended.

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### Age Estimation, Fourth Rib, Pubic Bone

## A128 Application of the forAGE Program to Pubic Symphyseal Surface in a Korean Population

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**Learning Overview:** After attending this presentation, attendees will understand what points need to be considered when applying the *forAGE* program to a population other than European American, through the results of applying the *forAGE* program, an age estimation program that utilizes the 3D difference of the pubic symphysis surface, to the Korean population.

**Impact Statement:** This presentation will impact the forensic science community by presenting new results of estimating the age of Asian populations using the *forAGE* program and the limitations of age estimation programs designed with specific populations.

*ForAGE* is a program that estimates the age-at-death of human pubic symphysis based on 3D scans. This program was devised by Dennis E. Slice and Bridget F.B. Algee-Hewitt and based on the three different scores: Slice and Algee-Hewitt (SAH) score, Bending Energy (BE), and Ventral Curvature (VC).<sup>1,2</sup> However, these scores and age estimation regression equations were obtained through European American pubic symphysis.

Changes to the pubic symphysis surface by aging were evaluated as one of the most reliable indicators for estimating age. However, there has been no study conducted regarding the relationship between changes in the pubic symphysis and actual age and applying the age estimation method for a specific population among a Korean population. The purpose of this study is to compare the difference between the actual age and the estimated age in a Korean population to see if the *forAGE* program is applicable to other populations of different ancestral origin.

One hundred seven Korean pubic symphysis surfaces, donated from Yonsei University College of Medicine, (40 males and 21 females, age range 47–96 years, mean age 75.9 years) were used in this study. Specifically, the number of pubic symphysis surfaces was 4 people in their 40s, 6 people in their 50s, 6 people in their 60s, 39 people in their 70s, 35 people in their 80s, and 4 people in their 90s. The pubic symphysis surfaces were scanned using Arctic Space Spider scanner (Artec3D). The difference between the actual age and the estimated age was compared via the *forAGE* program.

First, the prediction equations using the SAH score in a Korean population showed the following results.<sup>1</sup> According to all pubic symphysis samples over 60 years of age, the estimated age was lower than the actual age. Interestingly, for those under 60 years, the estimated age was higher than the actual age. The Root Mean Square Error (RMSE) of the 60s, 70s, 80s, and 90s Korean age groups were 7.02, 18.32, 27.24, and 37.38, respectively. With aging, the difference between the actual age and estimated age became larger.

Second, similar patterns were found in the analysis of the new regression lines using VC, SAH score, and BE. The estimated age of all pubic symphysis sample, was lower than that of the actual age. Among the five new regression lines, the regression line with VC had the lowest RMSE. The RMSE increased in order of the regression line with VC&SAH, SAH, VC&BE, and BE. Below 60 years, the RMSE of new regression line with VC was 15.46, but 18.85 in 60 years, 33.54 in 70 years, 36.42 in 80 years, and 50.81 in 90 years. Surprisingly, the difference between the actual age and estimated age was greater in those aged over 70.

This investigation confirmed whether the age estimation method through a 3D scan of pubic symphysis can be applied to the Korean population. The *forAGE* was a study based on young and middle-aged people that showed large differences among the Korean population. The prediction equations using SAH score and new regression lines using VA are more accurate for the 50-year and 60-year groups. In the 50–60 age groups, *forAGE* age estimation can complement and improve the accuracy of other age estimation methods. However, this research for middle- and old-aged Koreans shows there is yet a need for further research to apply the equation to a wider variety of age groups.

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### Pubic Symphysis, Age Estimation, Korean

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## A129 Age Estimation From Sternebral Fusion in an Indian Population: A Computed Tomographic Evaluation

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the use of sternebral fusion in human age estimation.

**Impact Statement:** This presentation will inform attendees and the forensic science community of the utility of sternebral fusion in ascertaining whether an individual has attained the medicolegally significant ages of 16 and 18 years.

Forensic age estimation is a crucial aspect of the human identification process. It is routinely done in dead as well as living individuals and in civil as well as criminal cases. The most common methods of forensic age estimation include the study of epiphyseal fusion of the bones and development of the teeth. The human sternebrae are an example of skeletal indicators of age that can be used in human identification practices. Limited literature exists on their utility in estimating the age of individuals nearing the legal age of maturity.

The present study was conducted with the aim of assessing the utility of each of the sternebral fusion in age estimation and to ascertain if these fusion stages can help in identifying whether an individual has attained the medicolegally significant ages of 16 and 18 years. The present study investigated the extent of fusion of sternebrae 1–2, sternebrae 2–3, and sternebrae 3–4 in 148 study participants (74 females, 74 males) aged 10.06 years to 23.47 years by analyzing their thoracic Computed Tomography (CT) images. The age of male and female participants was evenly distributed—there were 10 males and 10 females in the age group of 10 to 12 years, there were 10 males and 10 females in the age group of 12 to 14 years, there were 11 males and 10 females in the age group of 14 to 16 years, there were 10 males and 10 females in the age group of 16 to 18 years, there were 11 males and 11 females in the age group of 18 to 20 years, there were 12 males and 11 females in the age group of 20 to 22 years, and there were 10 males and 12 females in the age group of 22 to 24 years. The fusion scores for these were found to be statistically significant ( $P < 0.001$ ) with sternebrae 1–2 fusion showing the highest coefficient of correlation at  $R=0.900$ . Simple and multiple linear regression models were generated using each of the sternebral fusions separately and together for estimation of age. The standard error of estimate for the regression models ranged from 1.51 to 2.86 years. Box and Whisker plots were constructed to see the fusion score wise age distribution of the study population, and it was observed that sternebral fusion has the ability to indicate whether an individual has attained the age of 16 and 18 years.

The present study concludes that sternebral fusion can act as an accurate method of forensic age estimation of juveniles and young adults and can be used by itself or as an adjunct to other established methods of age estimation in juveniles.

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### Forensic Anthropology, Age Estimation, Sternebral Fusion

## A130 Age Estimation Via Dry Bone Measurements: Evidence From Samples of Soldiers Exiled in Two Concentration Camps in Bari

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the contributions of mandibular dimensions measured on dry bone in estimating age at death from skeletal remains.

**Impact Statement:** This study will impact the forensic science community by offering an improved method of estimating age at death from mandibular measurements.

In cases where the identification of human remains is necessary as a result of accidents or in cases of criminal investigations or ethnic studies, the determination of age and sex is one of the first objectives sought by the medical examiner.

Recently, studies have highlighted that the anatomy and shape of the mandible change in proportion to age and sex.<sup>1</sup> In regard to age, a modification in the structure of the masticatory muscles was observed, which is manifested by a reduction in contractile strength.<sup>2</sup> Furthermore, sex differences in the morphology of the mandible are a result of different genetic patterns, with exponential growth during puberty observed for males, but not females.

This study analyzed three mandibular dimensions (gonial angle, bigonial width, and ramus height) as determinants of age for a group of soldiers of the Royal Yugoslavian-Chetnik Army in exile in Bari in 1941. The literature shows how these mandibular dimensions have been studied through radiological investigations (panoramic radiographs, computed tomography, cephalograms).<sup>3</sup> The goal of this research is to collect the three mandibular measurements (gonial angle, bigonial width, and ramus height) directly from the skull to further examine mandibular age-related dimensional variations.

The sample consisted of 93 mandibles of Yugoslav origin from the skeletal collection of the Institute of Legal Medicine at the University of Bari. The remains represent soldiers of the Royal Yugoslavian-Chetnik Army who lived at two concentration camps of the Bari area during the World War II. A single observer collected the three mandibular measurements. To assess reliability and reproducibility, a second observer recorded the measurements at a later date.

The analyses were performed using an Excel® database and a statistical analysis was performed using Statal 13MP software. Bivariate analysis was used to compare the study parameters with age. All independent variables with a p-value  $\leq 0.25$  have been considered suitable for the multivariate analysis. Multivariate logistic and linear regression models were used to correlate independent variables with age. The results of the multivariate analysis were expressed in 95% Confidence Intervals (CIs), with a statistically significant level of p-value 0.05.

Statistically significant differences in the bigonial width and gonial angle were noted in this study according to the age of the individuals, while the ramus height was not statistically correlated with age.

In conclusion, this research confirms that the mandible is important for age determination and these findings showed statistically significant differences in bigonial width and gonial angle measurements according to the age of a sample of soldiers who lived inside the two concentration camps of the Bari area during the World War II. The ramus height dimension was not statistically significant with age. The greatest strength of this study, however, is the absence of geometric distortions of radiography present in other studies, due to direct measurements performed on the mandibles. Thus, knowledge of these different patterns is essential in determining the age of human skeletal remains, and the morphometric analysis of mandibular dimensions can be a helpful tool in forensic science.

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### Forensic Anthropology, Identification of Human Remains, Concentration Camps

## A131 Developing Subadult Sex Estimation Standards Using Adult Morphological Sex Traits and an Ontogenetic Approach

Stephanie Cole, MS\*, University of Nevada-Reno, Reno, NV

**Learning Overview:** After attending this presentation, attendees will understand how subadult sex estimation can be achieved using traditional adult morphological sex traits within an ontogenetic framework.

**Impact Statement:** This presentation will impact the forensic science community by providing a unique approach to subadult sex estimation that is as accurate as methods used for adults.

Morphological traits of the skull and pelvis are the most relied upon for estimating sex from the human skeleton. Currently, these traits are typically only applied in individuals estimated to be 18 years of age and older. However, these traits are associated with puberty and, therefore, should be applicable in individuals prior to adulthood. Recent research has shown that dimorphism in the morphological traits of the skull and pelvis is expressed as early as 13 years of age.<sup>1,2</sup> Further, ossification of the iliac crest is associated with menarche in females and, therefore, can serve as a skeletal indicator of puberty, thus providing insight into the status of skeletal maturity without needing to rely on an age-at-death estimate.<sup>3-5</sup>

A new method for estimating subadult sex is currently being developed using an ontogenetic framework. The method utilizes the most frequently relied upon adult morphological sex traits of the skull and pelvis, but with reworked trait definitions and ordinal scoring procedures that are more appropriate for use with immature remains. The skull traits include: mastoid process, glabella, supraorbital margin, nuchal crest, and mental eminence. The pelvic traits include: ventral arc, pubis morphology, subpubic contour, medial aspect of the ischiopubic ramus, obturator foramen, greater sciatic notch, and preauricular sulcus. Additionally, the method incorporates skeletal indicators of puberty, such as the iliac crest, to assist practitioners in deciding whether an individual is mature enough for sex estimation to be attempted.

The modified traits were scored using a sample of 292 American individuals (m=165, f=127) aged 8 to 21 years from the Subadult Virtual Anthropology Database. Random forest was used to develop predictive models to estimate sex. A subset of individuals was then assessed for stage of iliac crest development and only individuals exhibiting ossification and/or fusion were retained (n=154, m=72, f=82, age=10-21yrs). A second round of random forest models was then employed to determine if accuracy improved.

When all individuals were included in the analysis regardless of iliac crest development stage, the combined sex accuracy was 92.8% with a large sex bias in favor of male classification (m=96.9%, f=87.4%). Out of 21 misclassified cases, 18 had an unossified iliac crest, and two of the three additional misclassifications were individuals younger than 13 years of age. When only individuals with an ossified and/or fused iliac crest were considered, the combined sex accuracy was 97.4% with a small sex bias in favor of male classification (m=100%, f=95.1%). Three out of four misclassified cases were individuals aged 11 years, with one case exhibiting minimal ossification.

The high classification rates demonstrate that adult morphological sex traits can be accurately applied to individuals that have yet to reach skeletal maturity when using an ontogenetic approach. The balanced, and higher, classification accuracy obtained when stage of iliac crest development was considered suggests that skeletal indicators of puberty provide a valid way to capture skeletal maturity without the need to rely on an age-at-death estimate.

Additional skeletal indicators of puberty are being incorporated and additional data will be collected from the United States and abroad. The finalized method is anticipated for 2022 and will be available as a graphical user interface, which will be freely accessible across multiple platforms, including MorphoPASSE and KidStats.

*This research was funded by the Forensic Sciences Foundation (Lucas Grant) and the National Institute of Justice (2015-DN-BX-K409, 2020-R2-CX-0024).*

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### Sex Estimation, Juvenile, Puberty

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## A132 Investigating Reproducibility of a Skeletal Age Estimation Method on Two Samples With Remarkably Different Demographic Profiles

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**Learning Overview:** After attending this presentation, attendees will have a more nuanced understanding of how composition of the reference sample as a methodological issue can constrain age estimation of human skeletal remains.

**Impact Statement:** In situations in which age estimation is used as evidence in a court of law, methods used should be reliable according to *Daubert's* admissibility standards. Careful examination of their reproducibility will produce more robust evidence overall.

While age estimation methods are routinely applied in biological anthropology, investigating their reliability has never been straightforward. Typically, a method is considered reproducible if agreement is over 80% or Cohen's Kappa is "substantial" ( $\kappa > 0.6$ ).<sup>1</sup> Yet, investigating the validity of these boundaries has not been carried out in detail. Among other issues, these parameters do not consider key aspects, such as the demographic profile of the study sample.<sup>2</sup>

The present research attempts to investigate how reproducibility of the same method is influenced by tests in different samples. To this end, two intra-observer repeatability trials were compared. This involved scoring of 25 morphoscopic traits on the pubic symphysis of 29 adult human remains from the Carlos Germann Collection curated at Simon Fraser University and 30 individuals from the Terry Collection at the Smithsonian Institution.

In the former sample, age-at-death ranged from 52 to 92 years, with an average of 75.4 years. Results indicate that agreement was 84% on average and Cohen's kappa was substantial ( $\kappa=0.63$ ). On the contrary, the sample from the Terry Collection consisted of individuals aged 23 to 97 years with a lower mean value at 57.6 years. Results of this trial showed virtually the same degree of reproducibility, with 85% and substantial Cohen's kappa agreement ( $\kappa=0.67$ ). Both tests were performed by the same observer, separated by a period of two years.

However, the demographic profile of both samples was fundamentally different. The trial using the Terry Collection was performed on a strategically selected sample, which ensures maximum representativeness of all age-related variation.<sup>3</sup> This implies a broader range of scores is to be expected in each feature. On the other hand, the sample from Simon Fraser University was not carefully selected. Instead, all available bone fragments in the collection were examined, which is not an uncommon practice in bioanthropology. Being this is a limited range of ages-at-death, there is potentially less variation in each feature. This would undoubtedly make the observer more likely to agree on given scores. Despite this fundamental difference, intra-observer agreement results are virtually equivalent and meet all standards.

Overall, details about demographic composition of the test sample are essential for assessing reproducibility of a method. However, this information is often not disclosed in bioanthropological literature. In light of this issue, more error tests are needed to assess the impact of observer bias in age estimation of human skeletal remains.

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### Biological Anthropology, Age Estimation, Human Variation

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## A133 A Revised Method for Adult Age-at-Death Estimation From the Auricular Surface of the Ilium

*Krista Bennett, MA\**, Louisiana State University, Baton Rouge, LA; *Sophia Reck, MA*, Louisiana State University, Baton Rouge, LA; *Teresa Wilson, PhD*, Louisiana State University, Baton Rouge, LA

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of age-related features on the auricular surface of the ilium that may assist with providing more accurate age estimations for individuals of advancing age.

**Impact Statement:** This presentation will impact the forensic community by providing preliminary results from the application of a revised Buckberry and Chamberlain (BC) adult age-at-death estimation method using the auricular surface of the ilium.<sup>1</sup>

The original BC method used assessments of five traits on the auricular surface of the ilium surface. The lead investigator scored each of the 104 ox coxae from the William Bass Donated Collection using the original five traits from the BC method as well as retroauricular activity and bone quality. The combined seven traits were used to produce a new set of composite scores with nine corresponding age phases. The resulting age phases were then evaluated for an association with known age-at-death and compared to the original BC method. Spearman's rho denotes a high correlation between age and phase ( $r=0.821$ ;  $p<0.01$ ) for the revised method, as compared to the moderate correlation between age and phase for the original BC method ( $r=0.692$ ;  $p<0.01$ ).

To address inter-observer error, three investigators independently scored 29 auricular surfaces from the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Donated Skeletal Collection in the blind using the revised method. Cohen's Kappa was used to measure agreement between the three investigators ( $\kappa=0.095-0.223$ ). While the inter-observer agreement was low, the accuracy of the age intervals associated with the nine age phases was high. For age-at-death estimations, 79%–93% of the specimens age-at-death fell within the three investigators assigned phase age intervals.

The goal of this project was to determine if including two additional age-related features of the auricular surface of the ilium could increase the ability to provide accurate age-at-death estimation. The preliminary results are promising, suggesting that integrating assessments of retroauricular activity and bone quality produce a stronger correlation between age and phase for the features of the auricular surface than previously observed. The low inter-observer agreement, however, implies further clarification is needed for all scorable features to allow for greater repeatability of the revised method.

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### Forensic Anthropology, Age Estimation, Auricular Surface

## A134 The Application of Computed Tomography (CT) -Rendered Images for the Evaluation of Traits Associated With Ancestry Estimation in Children

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**Learning Overview:** After attending this presentation, attendees will have an improved understanding of the reliability of using a digital approach to the examination of juvenile remains.

**Impact Statement:** This presentation provides the forensic science community with data on the reliability of visualizing morphological traits often used in ancestry estimation on CT scans of a juvenile sample.

Very few studies have been able to show good accuracy rates for ancestry estimation in adults. No research has been able to assess the accuracy of ancestry estimation in children, even though a few studies have suggested that features associated with ancestry are stable across the prenatal and postnatal periods.<sup>1</sup> While we are of the opinion that ancestry estimation is plagued with unsurmountable methodological and theoretical problems and may, in fact, be ineffective and futile, in practice the reliability of different visualizing techniques for the purpose of ancestry estimation is important to assess when engaging in a critical investigation of ancestry.<sup>2</sup> This is particularly true given the increasing availability of CT scans and 3D rendering methods for research purposes, which are considered reasonable proxies for visualizing and measuring dry bone for a variety of goals.<sup>3,4</sup> This study questions whether 3D rendering of CT scans can be used to accurately visualize the six traits used in the Optimized Summed Scored Attributes (OSSA) method. The OSSA method has been studied and used for adults of Black and White ancestry.<sup>5,6</sup>

For this study, 30 CT scans of individuals from 0 to 19 years of age were selected from the New Mexico CT collection. The sample is equally split between males and females. Only individuals listed as “Black” or “White” were chosen for this study. Cause of death was considered to avoid selecting individuals with trauma to the head or face area, which could obscure the visibility of the traits.

Scans had a 1mm slice interval and were rendered using RadiAnt 2021.1 DICOM viewer software. While the scans were preselected based on the above criteria, scoring was done blind, without knowing case demographics.

The sample was divided into five age groups (0-2.9, 3-6.9, 7-9.9, 10-14.9 and 15-19.9 years) with six individuals per group. Results indicate a 70% accuracy overall, comparable to studies using adults and dry bone.<sup>6</sup> While the results seemed increasingly consistent with the recorded ancestry with increased age, the 3-6.9 age group showed a 16% accuracy rate. In the oldest age group, the results were 100% consistent with the recorded ancestry. While this may suggest improved accuracy closest to adult age, this sample includes a majority of “White” individuals, resulting in low (73% in “Whites”) to very low (50% in “Blacks”) ancestry-specific accuracy rates.

Some features presented difficulty. An OSSA score of 0 defines features considered more common among “American Blacks” and 1 among “American Whites.” Nasal bone sutures were almost universally challenging to discern. This introduces some uncertainty into scoring the Nasal Bone Structure (NBS) feature. If the difference between an oval or low/round NBS (OSSA score 0) is indiscernible from NBS exhibiting a plateau or triangular morphology (OSSA score 1), the overall score will be impacted. The Inferior Nasal Aperture (INA) is another potential source of error in younger individuals since the nasal sill feature may be smaller than the slice interval. The presence of a sill differentiates an OSSA score of 1 from 0. Both of these may result in a different outcome.

This research suggests that 3D-rendered CT scans of juveniles can be reliably used to visualize traits included in the OSSA method. However, this visualization confirms the generally poor to very poor accuracy of the method and highlights the difficulty in scoring the traits, particularly in smaller individuals whose features are smaller than the slice interval. A critical investigation of ancestry estimation can reliably use CT scan technology as long as it has proper consideration for the limitations of the technology.

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### Children, Ancestry, Computed Tomography (CT)

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## A135 Geometric Morphometrics for Sex Estimation of the Scapula

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the use of scapular Linear Measurements (LM) and Geometric Morphometrics (GM) for sex estimation.

**Impact Statement:** This presentation will impact the forensic science community by describing the accuracy in sex estimation obtained from LM versus GM assessment. This is significant as estimating the biological profile of the individual is one of the main tasks of the forensic anthropologists' casework.

When forensic anthropologists are called to consult on casework, there is an identification hierarchy that includes estimating four parameters of the biological profile (age, sex, stature, and ancestry). Sex estimation can significantly increase chances for positive identification of deceased individuals, reducing the missing persons pool by approximately 50%. Skeletal elements may be easier to classify if they have a sexually dimorphic appearance, meaning there is a distinct and consistent difference in size or shape between the females and males of a particular species. If the deceased individual is left in a context that makes them vulnerable to various environmental factors, it is likely that the skeleton may be incomplete during recovery. Current forensic anthropological literature focuses primarily on the skull for sex estimation when the pelvis is not available, excluding the use of postcranial elements for these methods.

To address this gap, this project explores scapular morphology through both traditional LM and GM. Traditional LMs are more influenced by size variation, while GM, a quantitative method that captures geometry rather than just the isolated linear distances, is expected to be more efficient at capturing and detecting shape differences. The formal hypothesis for this project was that, since the pelvic girdle morphology relates to both shape and size variations within females and males, the same could be expected from the shoulder girdle. Therefore, this study expected the GM to be more efficient at discriminating sex from the scapula than the LM method, as the former captures shape (geometry) more efficiently. Data for this project were obtained from the Bass Collection ( $n=106$ ) to assess the GM method, and from the Forensic Databank ( $n=1,252$ ) to assess the LM method.

Linear Discriminant Analyses (LDA) were conducted on two LMs (scapular height and scapular breadth), as well as on the Principal Component Analysis (PCA) obtained from the GM analysis. Overall, the Correct Classification Rate (CCR) for sex estimation using LR was 93.1%. The CCR for the GM analysis was only 69.9%. Sex estimation from the linear measurements was therefore more accurate, contradicting the formal hypothesis. This indicates that, unlike in the pelvic area, the most significant scapular variation between females and males, as measured in this study, appears primarily related to Sexual Size Dimorphism (SSD). However, due to the limited array of landmarks selected for this study, the resulting rough, overall scapular shape would fail to detect subtle or localized sexual shape differences, and thus classification through GM would likely produce better results when a larger number of scapular landmarks are defined and considered in the analysis.

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### Sex Estimation, Geometric Morphometrics, Biological Profile

## A136 The Impact of Sex and Population Affinity on the Dental Development of Subadult Individuals

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**Learning Overview:** After attending this presentation, attendees will understand the impact of sex and population affinity on subadult age estimation using dental development within a framework of Transition Analysis (TA).

**Impact Statement:** This presentation will impact the forensic science community by quantifying the impact of sex and population affinity on age estimation following the Moorrees and colleagues method of dental development within the statistical framework of TA.<sup>1</sup> This framework attempts to estimate age based on a multitude of skeletal features, which allow for age estimates that are unique to each individual. Estimates from TA include a maximum likelihood estimate, which is expressed as a point age, and confidence intervals at varying levels, which are an age range. By combining developmental traits into one age estimate, several issues can be addressed and resolved, including the ability to use multi-feature phases with fixed age intervals, solving the reliance on open-ended age categories, and reference sample mimicry.<sup>2,3</sup> When estimating age, dental development is preferred as the timing and growth of human dentition is heavily regulated by genetics, with less influence from environmental factors.<sup>3,4</sup> As such, age estimated from tooth root and crown development is generally accepted as more accurate than those based on skeletal features.

Radiographs from 189 individuals (males=94; females=95) from the University of New Mexico Orthodontics case file system (UNM; <https://searchorthodontics.health.unm.edu>) were examined for this project. Individuals ranged in age from 6 to 18 years old, and comprised five ancestral groups: American Black ( $n=26$ ), American Indian ( $n=32$ ), American White ( $n=65$ ), Asian ( $n=15$ ), and Hispanic ( $n=51$ ). Age cohorts were divided into one-year groups. At a minimum, two individuals were used per age cohort. Each mandibular tooth was assigned a score ranging from 1-14 for permanent dentition and 1-17 for deciduous dentition to account for resorption following Moorrees and colleagues original method.<sup>1</sup> From these observations, age estimations were generated using the TA code modified by Kamnikar and colleagues.<sup>5</sup> Maximum likelihood point age estimates and age ranges at the 90% confidence interval were generated for each individual.

Results show that males and females follow a consistent developmental trajectory between estimated dental age and chronological age. Average female inaccuracy was, on average, 12 months, and average male inaccuracy was 10 months. All populational affinities analyzed following along a consistent trajectory between estimated age and chronological age. For age six cohort, populational affinity differences varied from 36.6 months for American White individuals and a low of 11.5 months for American Black individuals. However, these results could be due to multiple radiographs for one individual and a small sample size ( $n=1$ ) for another. In the age seven cohort, the highest variance between estimated and chronological age was 61.4 months for American Whites. The lowest variance was 2.8 months in Hispanic individuals. Ages 8 to 18 had variances less than one year among all population affinities, and some were so small (.96 months) as to be considered negligible. Because small sample sizes were utilized in this study, the results describing variation in estimated and chronological age among population affinities requires further investigation with a larger sample size by age cohort.

Prior research has determined that females mature before males, both skeletally and dentally. This research comparing estimated and chronological age using dental development in a framework of TA showed that females and males showed minimal variation between ages. This was also true for population affinity; however, reassessment with a larger sample size per age cohort is strongly recommended.

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### Forensic Anthropology, Age Estimation, Sex

## A137 The Correlation Between Spheno-Occipital Synchondrosis (SOS) Fusion and Pubertal Stage From Postmortem Computed Tomography (CT) Scans

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**Learning Overview:** After attending this presentation, attendees will have reviewed the correlation between the development of the SOS and pubertal stage after Shapland and Lewis.<sup>1,2</sup>

**Impact Statement:** This presentation will impact the forensic science community by presenting novel analysis of the correlation between the fusion of the SOS and pubertal stage.

Puberty is a significant life history event associated with developing fecundity.<sup>3</sup> The age of onset of puberty and how this is related to skeletal development is of particular interest to those that are tasked with age estimation, whether of the living or the dead, because it may shed light on patterns of variability that are widely documented and yet remain poorly understood.<sup>4,5</sup>

This study analyzed staged SOS and assessed pubertal scores from 322 postmortem CT scans to examine the possible correlation between the SOS and pubertal stage. The CT scans were obtained from the New Mexico Decedent Image Database, and are of individuals aged between 8 and 20 years (53% male).<sup>6,7</sup> Pubertal scores were assessed after Shapland and Lewis; the seven stages were prepuberty, initiation of puberty, acceleration, Peak Height Velocity (PHV), deceleration, maturation, and postpuberty.<sup>1,2</sup> SOS scores were adapted from Franklin and Flavel, these stages were unfused, fusing endocranially, fusing ectocranially, and fused.<sup>8,9</sup> Pearson's correlation test was performed using R version 4.1.0.<sup>10</sup>

Pearson's  $r$  was .798 ( $p < .000$ ) for females and .851 ( $p < .000$ ) for males. For the pubertal scores, the mean ages by stage (in years) for males were prepuberty=9.0 (standard deviation [sd]=1.20), initiation= 9.85 (1.07), acceleration=12.89 (0.99), PHV=14.24 (1.13), deceleration=16.33 (1.13), maturation=18.00 (1.29), postpuberty=19.02 (1.05), and in females were prepuberty=8.33 (sd=0.58), initiation= 9.14 (0.69), acceleration=12.07 (2.28), PHV=13.77 (1.96), deceleration=16.10 (1.81), maturation=18.03 (1.18), postpuberty=19.22 (1.28). For SOS scores, the mean ages by stage were unfused=11.24 (2.43), fused endocranially=13.86 (1.90), fused ectocranially=15.69 (1.49), fused=17.80 (1.93) for males and unfused=10.00 (1.75), fused endocranially=12.86 (2.73), fused ectocranially=13.67 (2.52), fused=17.65 (2.19) for females.

A statistically significant correlation between the SOS and pubertal stage is presented here. The slightly lower correlation between SOS stage and pubertal stage in males could indicate that the development of SOS is less sensitive to the effects of environmental factors that affect puberty. A larger study with environmental factors (such as socio-economic and nutritional status) included could yield support for this hypothesis.

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### Forensic Anthropology, Age Estimation, Pubertal Age

## A138 A Comparison of Frontal Sinus Morphology Using Scout Radiographs and 3D Skull Images: Implications for Forensic Identification Methods

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**Learning Overview:** After attending this presentation, attendees will better understand the effect of image modality on basic frontal sinus features.

**Impact Statement:** This presentation will impact the forensic community by illustrating that practitioners can combine modalities (radiographs and images derived from Computed Tomography [CT] scans) when visually assessing frontal sinus features for identification purposes.

Currently there is a general acceptance that frontal sinus morphology can be used as an identification method when comparing antemortem and postmortem images. Frontal sinus comparisons are commonly completed via visual assessment, although several quantitative methods have been developed in consideration of the *Daubert* standards. All methods, however, are influenced by certain basic frontal sinus features, such as number of arcades. While most frontal sinus identification studies utilize antemortem and postmortem samples of the same imaging modality, in practice, an antemortem CT image may need to be compared to a postmortem radiograph. The goal of this preliminary study is to assess whether basic frontal sinus features can be observed consistently across image modalities.

This study incorporates scout radiographic images and CT scans from 30 individuals from the New Mexico Decedent Image Database.<sup>1</sup> Translucent models from the CT scans were created to view the frontal sinus, oriented to match their scout images, and saved as 2D images. A different observer then assessed frontal sinus morphology for both the scout radiographs and CT-model images. First, sinus Presence/Absence (P/A) was established, with presence being above the supraorbital line. If present, the following variables were recorded: intersinus septum (P/A), right sinus (P/A), left sinus (P/A), number of arcades for right and left sinuses separately, and number of total arcades. Due to difficulties in counting arcade number, a range was recorded with minimum and maximum counts.

Statistical analyses were run in SPSSv25 with significance set at 0.05. Non-parametric McNemar's tests were used to investigate differences in binary (P/A) traits between paired scout and CT-model images, while Wilcoxon Matched Pairs Signed Ranks tests were employed for continuous traits (arcade number). Results of the McNemar's test showed that P/A of the intersinus septum ( $p=0.250$ ) and right/left sinus ( $p=1.000$ ) were consistent across the two modalities. The Wilcoxon tests also revealed no significance differences in scoring between image modalities. Z-scores indicated that the minimum and maximum total arcade counts were scored with higher consistency, though the overall estimated error was slightly lower when scoring the left and right sides independently (e.g., the estimated percent error for total arcade = 6.79%, left arcade = 5.07%, right arcade = 4.06%).

Although some variables were more consistent than others, the overall results of this study indicate that utilizing different image modalities should not alter assessments of basic frontal sinus morphology—as long as the images are orientated similarly. This provides preliminary support for mixed-modality frontal sinus comparisons. Future research should be conducted on larger sample sizes to test how reliability and accuracy rates vary for additional variables (e.g., intra-sinus septa) and ways to address scoring issues in low-quality images (as encountered when identifying intersinus septa and arcade numbers). Deviations in scoring, either due to image quality or modality that affect features such as arcade number, could negatively impact identifications, particularly when quantitative coding methods are employed.

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### Forensic Anthropology, Image Modality, Positive Identification

## A139 The Relationship Between Environmental Factors and Skeletal Metrics in Contemporary New Mexicans

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**Learning Overview:** After attending this presentation, attendees will better understand the differential effects of environmental factors on postcranial skeletal metrics across sex and sociocultural groups.

**Impact Statement:** This presentation will present an assessment of whether socioeconomic and environmental variables have differential effects on skeletal metrics across sex and sociocultural groups that may affect the accuracy of the use of postcranial skeletal metrics when constructing a biological profile.

The biological profile is a fundamental portion of a forensic anthropology report, aiding law enforcement in the identification of skeletal remains. Stature and sex estimations can be performed with a high degree of accuracy from postcranial skeletal metrics, and this accuracy increases when ancestry is taken into consideration.<sup>1-6</sup>

Negative effects from tobacco and substance use on skeletal health have been widely documented by the medical community, including the reduction of bone mineral density in tobacco and alcohol users.<sup>7-9</sup> Additionally, health outcomes are often correlated with the socioeconomic status and perceived race of individuals in the United States.<sup>10</sup> Within the law enforcement community, race is often utilized interchangeably with population affinity or ancestry. However, race is a cultural construct and is variably associated with the actual genetic similarity of individuals within a population. Because social categories impact the life experiences of individuals, incorporating these categories into sex and stature estimation equations may improve predictions because of the sociocultural environmental effects that potentially significantly differ among groups. These factors are often not considered in the interpretation of skeletal metrics within forensic anthropology. Therefore, the intersection between environmental factors and perceived race within the United States must be thoroughly investigated to assess if the performance of commonly used stature equations and sex estimation methods derived from postcranial metrics may be significantly affected. This pilot study tested the assumption that socioeconomic and environmental variables have differential effects on skeletal metrics across sex and socioculturally-defined groups.

The sample consisted of 147 whole body Computed Tomography (CT) scans from the New Mexico Decedent Image Database (NMDID) of individuals between 20–55 years of age, listed as “White” ( $n=42$  female, 43 male) and “Hispanic” ( $n=33$  female, 29 male), with recorded demographic information.<sup>11</sup> Environmental factors included in this study were tobacco use, alcohol use, childhood socioeconomic status, and history of strenuous lifting. Maximum diaphyseal measurements were taken from five long bones as well as bi-iliac breadth using RadiANT DICOM™ viewer.<sup>12</sup> Additionally, living height was obtained from the NMDID records. The data were analyzed using basic functions of the R statistical language.<sup>13,14</sup>

Two multivariate linear regression models relating living height and skeletal metrics to demographic and environmental covariates were fitted. The null model only included the additive effects of sex, sociocultural group, year of birth, and environmental factors, while the full model incorporated the interactions of sex and sociocultural group with each of the environmental factors. An Analysis of Variance (ANOVA) was conducted to test if the interactions distinguishing the null and full models are statistically supported. The results indicate that differential effects across sex and sociocultural group are present (Pillai’s Trace=0.59), but do not meet standard levels of statistical support ( $p=0.22$ ). This suggests that simple, non-population-specific adjustments for contemporary environmental exposures would be adequate for stature and sex estimates. However, it is possible that the ANOVA failed to reach significance due to the small size of the sample. Univariate model summaries do suggest variable significance of interactions with sociocultural groups. For example, the interaction of sociocultural group and alcohol use and the interaction of sociocultural group and childhood socioeconomic status meet conventional, uncorrected significance thresholds ( $p < 0.05$ ) as predictors of bi-iliac breadth, indicating that further investigation with a larger sample is warranted.

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## Skeletal Metrics, Environmental Factors, Biological Profile

## A140 A Validation of Compatibility of the Median Sagittal Plane for 3D Metric Analysis of Facial Models: Cone Beam Computed Tomography (CBCT) and Multidetector Computed Tomography (MDCT)

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**Learning Overview:** After attending this presentation, attendees will have gained an understanding of a measurement system designed for CBCT-based 3D models and its comparison with an MDCT measurement system.

**Impact Statement:** This presentation will impact the forensic community by providing information on the compatibility of two measurement modalities. With the increasing usage of cone-beam computed technology, a measurement system for CBCT-based models had been designed and its potential of exchangeability with the MDCT measurement system was tested.

The usage of CBCT in anatomical and anthropological studies has arisen.<sup>1</sup> Unlike MDCT using a fan-shaped beam, CBCT is an imaging modality taking a cone-shaped beam. Compared to MDCT, CBCT provides low radiation exposure and cost-effectiveness but a limited Field Of View (FOV). In cases of facial image acquisition, CBCT-based images could omit the occipital region due to smaller scanning areas.

The median sagittal plane of the measurement system for MDCT-based models was defined by three landmarks: nasion, prosthion, and lambda. The landmarks were selected to reflect the general anteroposterior shape of the skull. CBCT-based models, on the other hand, cannot utilize landmarks in the occipital region. Therefore, the median sagittal plane of the measurement system for CBCT-based models was defined with the nasion, prosthion, and bregma. It was necessary to validate the compatibility of standard planes due to the difference in the landmark composition of median sagittal planes.

This study analyzed 40 models of Korean adult males and females in total, with a random selection of five models in each decade of ages, from the 20s to 50s. This study measured the angle between standard planes using lambda and standard planes using bregma, and distance from landmarks and to each standard plane to measure the error between the CBCT measurement system and the MDCT measurement system. The landmarks were median landmarks (vertex, glabella, rhinion, nasospinale, gnathion) and lateral landmarks (frontotemporale, frontomalare temporale, euryon, zygon, midmasseter, gonion).

The average angle between MDCT standard planes and their counterpart CBCT standard planes were  $2.50 \pm 2.02$  degrees for median sagittal planes,  $2.49 \pm 2.01$  degrees for coronal planes, and  $0.29 \pm 0.31$  degrees for transverse planes. The distance between landmarks and standard planes was observed by paired *t*-test to verify whether the difference of standard planes exists. There was no statistical significance of the difference between MDCT standard planes and CBCT standard planes ( $p < 0.05$ , bilaterally).

The results exhibit that the CBCT measurement system is compatible with the previous MDCT measurement system. It would be preferable, however, to opt for lambda versus bregma with a subject with obvious facial asymmetry. The newly defined CBCT measurement system, nonetheless, will be useful for cases with incomplete scanning or damages of the occipital region.

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### 3D, CBCT, MDCT

## A141 A Validation of the Utilization of Automatic Placement of Anatomical and Sliding Landmarks on 3D Models for Shape Analysis of Human Pelves

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**Learning Overview:** After attending this presentation, attendees will better understand the utilization of automatic placement of anatomical and sliding landmarks on a 3D model for shape analysis of the human pelvis.

**Impact Statement:** This presentation will impact the forensic science community by suggesting the utilization of accurate 3D automatic anatomical information extraction and advanced statistical analysis for estimation of sex from whole and fragmentary pelves.

Identification of unknown persons is a challenge in South Africa. For example, in 2014/2015, approximately 1,300 bodies were left unidentified in Gauteng mortuaries.<sup>1</sup> In response to this crisis, The Forensic Anthropology Research Centre's (FARC) forensic anthropologists collaborated with the South African Police Service (SAPS) to analyze skeletal remains and provide estimations on sex, population affinity, stature, age, and traumatic injury to bone and pathology. Sex estimation techniques focus directly on the pelvis's morphological variation and the long bones' metric variation.<sup>2</sup> However, the pubic bone, the most sexually dimorphic feature on the pelvis, is not often recovered intact. Therefore, it is necessary to improve our abilities to extract biologically meaningful information from other parts of the pelvis. To this end, in the past three years, researchers at FARC have become involved in developing morpho-phenotyping shape analysis tools using automatic landmarking and semi-landmarking for forensic case analysis.<sup>3</sup> The purpose of this research was to validate the use of the automatic placement of anatomical and sliding landmarks shape analysis of the human pelvis using Computed Tomography (CT) scans.

CT scans of 88 adult pelves (44 females and 44 males) from South Africans were collected from the Steve Biko Hospital, in Pretoria, South Africa. The 3D reconstruction generations were performed using the Avizo® 2019.3 software and pelvis shape data information was acquired using the MeVisLab v. 2.7.1 software. The automatic placement of anatomical and sliding landmarks approach used in this research is adapted from the procedure recently published by Ridel and colleagues.<sup>3,4</sup> 3D reconstructions and anatomical and sliding landmark datasets were acquired using a manual and automatic approach and compared using reproducibility testing and Geometric Morphometric (GMM) analysis. To this end, 18 anatomical and 256 sliding landmarks (curve 1: *right greater sciatic notch*; curve2: *left greater sciatic notch*; curve3: *right ischiopubic region*; curve3: *left ischiopubic region*) were registered on 88 3D models of the same individuals using a manual and an automatic placement.

The anatomical and sliding landmarks reproducibility testing demonstrated minimal dispersion errors (<2mm) for both landmark datasets. The variance analysis showed that pelvis shape variation was statistically significant ( $p < 0.001$ ) for the sex variable among all configurations collected using both procedures, including subspecific discriminate shape matrices separately. Furthermore, cross-validated linear discriminant function analysis yielded an accuracy between 84.09% and 97.72% using an automatic method and between 65.90% and 93.18% using a manual approach.

Several studies using GMM to analyze pelvic-related shape variation for sex estimation concluded that GMM and multivariate statistics are a reliable method to quantify pelvic shape differences between the sexes.<sup>5</sup> The utilization of GMM for sex estimation using the pelvis is preferable to metric methods because they retain the objects' geometry and analyze subtle differences among structures.<sup>6</sup> Furthermore, GMM could significantly overcome the exclusion of fragmented pelves in the sex estimation analysis by creating discriminate shape matrices to analyze specific patterns not readily observed with a morphological approach. In a real-life application, using 3D imaging approaches is more reliable and efficient than traditional methods. However, researchers need a practical 3D scanning modality, such as a surface scanner, and an approach to extract 3D anatomical information, such as automatic landmark placements, along with advanced statistical analysis. All three of these tools will permit forensic anthropologists to estimate the sex from fragmentary remains in a more accurate and repeatable way.

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### Anatomical and Sliding Landmarks, Automatic Landmarking Approach, Sex Estimation

## A142 A Validation Test of the Total Difference Technique for Assessing the Frontal Sinus

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how to accurately and efficiently apply the Total Difference technique for assessing the frontal sinus in personal identification.

**Impact Statement:** This presentation will impact the forensic science community by increasing evidentiary standards through the refinement and validation of an objective technique in frontal sinus identification.

While several quantitative methods evaluating frontal sinus morphology for positive identification exist, few have been rigorously tested for validity and repeatability following *Daubert* standards. This study specifically tests the reliability of the Total Difference technique for evaluating frontal sinus outlines.<sup>1</sup> This technique determines sinus uniqueness through 59 linear measures collected from the origin (cranial midline) to sinus outline at 3° intervals. While informative, this process can be time-consuming; thus, the current study also investigates whether measurement aids (i.e., an overlay) and/or semi-automation macros increase efficiency and repeatability.

Following Cox et al., frontal sinus outlines were collected on 244 adult radiographs from the American Association of Orthodontists Foundation (AAOF) Craniofacial Growth Legacy Collection in ImageJ.<sup>1-3</sup> A randomly selected final sample (n=10 bilateral outlines, 590 data points) was required for significance testing with a 95% confidence level and 4% margin of error. Three methods for collecting the 59 linear measurements were tested: the original method's freehand; use of a 3° overlay; and a semi-automated macro. All radiographs were scaled by orbital breadth using the global average from Howell's dataset.<sup>4</sup> Two observers collected data using the overlay and semi-automated macro; observer two collected a subsample using the original freehand method without an overlay, and observer one collected a second round of data two weeks after the first using the overlay and semi-automated macro. Methods for collecting measurements were evaluated on the ability to reproduce all 59 angles consistently, efficiency of data collection, and consistency in origin placement (measured as a midline ratio). Analyses ( $\alpha=0.05$ ) were conducted in R.<sup>5</sup>

Time across the three modes was significant ( $p<0.05$ ): the freehand mode averaged 11.4 +/-4.1 minutes, the overlay mode averaged 8.9 +/-2.6 minutes, and the semi-automated mode averaged 6.7 +/-0.92 minutes. The maximum standard deviation of all midline ratios was 0.023, suggesting origin placement was consistent among and between all modes and observers. Placement of all 59 degrees had low overall bias (-0.04 +/-0.47) and inaccuracy (0.30 +/-0.36). There were significant differences between observers ( $p<0.05$ ), and significance reached  $p<0.001$  for both bias and inaccuracy for the overlay compared to the semi-automated method, as well as for the freehand method compared to either the overlay or semi-automated method; inaccuracy in the overlay method was greatest. Differences in lengths were significant between the overlay and semi-automated methods (bias, inaccuracy  $p<0.001$ ) and between the freehand and semi-automated methods (bias, inaccuracy  $p<0.001$ ). When results from observers were compared, inaccuracy was significantly different ( $p<0.001$ ). Greater differences in length were observed in sinuses with convoluted outlines where minor deviations in angle precision or origin placement influenced the distal termination of a line (i.e., the furthest point of the outline at that angle extended into the adjacent arcade). Further differences could stem from placement of the orbital breadth scalar, which artificially affects linear dimensions.

These results suggest application of the Total Difference technique warrants caution, particularly with complex sinuses and scaling methods. However, placement of the 59 linear measures is highly repeatable using any collection mode tested here and the semi-automated macro was the most efficient. Ongoing research will continue to evaluate this technique and its applications for personal identification and compare its performance with alternate methods for assessing frontal sinus individuality.

This project contributes to a grant from the U.S. Department of Justice, Office of Justice Programs, National Institute of Justice Research and Development in Forensic Science for Criminal Justice Purposes. (Award No. 2020-75-CX-0013).

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### Frontal Sinus Outlines, Personal Identification, Radiographic Comparison

## A143 The Precision of Standard Cranial Measurements in Dry Bones and Multi-Slice Computed Tomography (MSCT) Images: A Preliminary Study

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the issues that could arise from substituting documented skeletal collections with Computed Tomography (CT) images when developing standards for estimating biological profile.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence that cranial measurements from CT images may be equally valuable as direct skeletal measurements, but with particular caution for some cranial regions.

The osteometric analysis of skeletal remains is a fundamental step in reconstructing an individual's biological profile, especially when estimating ancestry, sex, and/or stature. The emergence of virtual anthropological methods bypassed the lack of documented skeletal collections but also raised the still unanswered question: Do the virtual measurements correspond to the physical ones? This question is essential when considering the requirements for admissibility of such evidence in court. Since those questions were still not completely considered, this study aimed to examine the repeatability of standard cranial measurements in a real and virtual environment.

The sample comprised 21 crania from the Early Medieval archaeological site Velim-Velišćak located in southern Croatia. CT scanning was done at the University Hospital Dubrava, Zagreb, Croatia, with an MSCT. Scanning parameters were 120kV and 320mA, respectively, with isometric slices using  $16 \times 0.75\text{mm}$  collimation. Digital Imaging and Communications in Medicine (DICOM) data files were imported into the Stratovan Checkpoint software, version 2018.08.07. This study included 31 standard cranial measurements from Data Collection Procedures for Forensic Skeletal Material 2.0. (DCP 2).<sup>1</sup> Sliding and spreading calipers were used to take measurements on bones, while MSCT measurements were obtained as interlandmark distances. Four observers took part in the study. Two observers took measurements on crania, and two placed landmarks on MSCT images. For each measuring method, one of the observers repeated measurements/landmarking. MCST Images were viewed in 2D (axial, coronal, and sagittal view) and 3D using semi-transparent 3D volume rendering. On each cranium, a total of 47 landmarks were placed using both 2D and 3D views. R (version 3.6.2), Rstudio (version 1.2.5033), and geomorph package were used to read landmarks, calculate interlandmark distances, and obtain 31 standard cranial measurements.<sup>2</sup>

A paired-samples *t*-test was used to examine intra-observer and inter-observer variability and differences between dry bone and "virtual" measurements. Technical Error of Measurement (TEM) and relative Technical Error of Measurements (rTEM) were calculated for measurements that showed statistically significant differences. rTEM values lower than 1.5% were considered acceptable for intra-observer error, while this threshold was set at 2.0% for inter-observer error.<sup>3</sup> The level of statistical significance was set at  $P \leq 0.05$  with Bonferonni's adjustment for multiple comparisons.

The results showed high intra-observer agreement both for dry bone and virtual measurements. High inter-observer agreement was reached on bones and images for most measurements, except for several measurements that include sutural landmarks and minimum frontal breadth. When the dry bone and virtual measurements were compared, they showed consistency in most of the cases. The most pronounced discrepancies were noted for mastoid measurements and breadth measurements.

Per research, this is the first study that examined cranial measurements from novel data collection standards both in a real and virtual environment.<sup>1</sup> The results showed that most cranial measurements were repeatable and relatively consistent regardless of whether they were measured directly on crania or on CT images. Unlike previous studies that mostly used 3D reconstructions only, this study also employed traditional radiological views, which probably positively impacted precision; however, due to the limited sample size and number of parameters considered, further studies are needed to obtain additional confirmations.

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### Craniometry, Virtual Anthropology, MSCT

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## A144 Uncovering Identities: A Case Study on Using Maxillary Sinus Morphologies in Human Identification

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**Learning Overview:** After attending this presentation, attendees will understand the potential of using morphological evaluations of maxillary sinuses for identification purposes.

**Impact Statement:** This presentation will impact the forensic science community by presenting an identification case study on eight individuals utilizing maxillary sinus morphologies.

**Introduction:** Human identification from skeletal structures is a significant forensic procedure, and visual comparisons of radiographic images taken antemortem and postmortem can be used as a source for identification.<sup>1</sup> Previously, in forensic contexts, maxillary sinuses have been examined using measurements and volumes.<sup>2</sup> Besides those approaches, the maxillary sinus shows high morphological variability and can be used for identification by examining morphologies on antemortem and postmortem radiographs. Morphologies are easily comparable, and the availability of suitable X-ray images is granted from dental practices, as dental overview images often additionally cover the maxillofacial area of the skull.

This study aims to test the method of morphological evaluation on maxillary sinuses for human identification on a real-life scenario.

**Materials and Methods:** For this study, a total of eight individuals were made available from two collections of the American Association of Orthodontists Foundation (AAOF) Craniofacial Growth Legacy Collections Project.<sup>3</sup> Within the framework of those longitudinal studies, radiographs were available of the same individuals on a semi-annual or annual basis to determine how the skull growths ranged from ages of 2 to 70 years.

For this study, an antemortem and postmortem situation was generated using two adult radiographic images. The first images, which simulate the antemortem dataset, were usually taken around the individual's 20<sup>th</sup> year of life, while the simulated postmortem image was taken later during the individual's life. Only standardized anteroposterior radiographic images were used for evaluations. Although the individuals were still alive at the time of the second radiograph, the passage of time during the images makes the images suitable for this case study.

In this study, method suitability was evaluated using elliptic Fourier Analyses. Correlations measuring Euclidean distances between the morphologies and neighbor joining clustering were applied to quantify method usability. The analyses were all performed and separated into left and right maxillary sinuses. Individuals with pathological changes in the maxillofacial area were excluded from the study.

**Results:** (1) Calculation of Euclidean distances between the morphologies for the antemortem and postmortem datasets show close Euclidean distances only for the antemortem and postmortem pair of one individual, while distances are greater between morphologies of different individuals. This is both visible in the right and left-sided morphologies. (2) Visualization through neighbor joining clustering validates the results.

With matching morphologies only between each antemortem and postmortem morphology of the same individual, this study supports and encourages the application of maxillary sinus morphologies on radiographic images for human identification.

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### Forensic Anthropology, Maxillary Sinus, Morphological Evaluation

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## **A145 The Sociodemographic Composition of a Modern Juvenile Postmortem Computed Tomography (CT) Collection: Examining Trends in Body Size, Manner of Death, and Demographic Parameters**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the make up of a modern postmortem CT sample, as well as the methods that can be used to examine and analyze sociodemographic composition in other similar populations.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility and value of examining trends in body size (weight and height), manner of death, and sociodemographic information (such as age, sex, race, and socioeconomic status) within modern juvenile forensic populations.

Body size, manner of death, and demographic data can be used to give researchers more in-depth and nuanced information, not only on the type of individuals who make up postmortem forensic samples, but also in predicting the composition of similar forensic populations in the future.

In this project, the forensic sample is composed of a subset of the New Mexico Decedent Image Database (NMDID). The individuals in the sample range from just under 1 year of age up to over 19 years of age and died between 2011 and 2017 in New Mexico.

A series of analyses were run to determine the frequency and proportion of each forensic variable that was listed in the database, including age at death, height (cm), weight (kg), Body Mass Index (BMI), manner of death, sex, race, and Hispanic ethnicity. Demographic information included in NMDID was obtained during the course of the death investigation, and additional information for some individuals was obtained from next-of-kin interviews. In addition, zip code information was used to pull socioeconomic information from United States Census data for the New Mexico region, including median income, poverty rates, and average educational attainment.<sup>1,2</sup> Forensic variables and sociodemographic variables were contrasted to examine relationships.

The goal of this research is to create a more complete picture of the individuals who make up the juvenile subsample of the larger NMDID. Understanding the types of children who are represented within this sample can give us a better understanding of who has a higher mortality risk based on their demographic and socioeconomic parameters. This type of analysis can be taken and applied to other places and contexts to examine similar trends among forensic populations.

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### **Juvenile Forensics, Demographic Analysis, Computed Tomography (CT)**

## A146 The Application of 3D Models in Age-at-Death and Sex Estimation of the Pubic Symphyseal Face

Anthony V. Lanfranchi, MS\*, Department of Applied Forensic Sciences, Mercyhurst University, Erie, PA; Luis L. Cabo, MS, Department of Applied Forensic Sciences, Mercyhurst University, Erie, PA

**Learning Overview:** After attending this presentation, attendees will understand the emerging applications of 3D models for the study of morphological variation contributing to biological profile estimation in forensic and biological anthropology.

**Impact Statement:** This presentation will impact the forensic science community by introducing the utility of high-definition 3D models in data collection and analysis of spatial and morphological data contributing to biological profile analysis in forensic anthropological applications.

The pubic symphyseal face provides one of the most reliable areas for age estimation from maturity to the mid fourth decade of age. Phase methods based on the evolution of the morphology of this articular surface with age, and in particular different variations of the Suchey-Brooks method, are among the most trusted and utilized by forensic anthropologists.<sup>1,2</sup>

However, quantifying the morphological traits utilized by traditional age estimation methods based on the pubic synchondrosis, in order to develop more objective metric methods also able to capture subtler morphological differences, beyond the rough ones between the more immediately identifiable traditional visual phases, has proven elusive. The most auspicious recent attempts to quantify the evolution of the pubic symphyseal face with age are based on capturing the geometry of the area from 3D scans, utilizing classic or modified Geometric Morphometry (GM) techniques.<sup>3-5</sup> The available of increasingly precise 3D scanners, at much more affordable prices than classic micro-Computed Tomography (micro-CT) scan systems, have made them popular in anthropometric applications, making this approach even more promising.<sup>6</sup>

Among these, the Artec Space Spider is a high-resolution 3D scanner that utilizes blue light technology to produce point accuracies in the  $\pm 0.1$  mm range, which has caused it to become a particularly popular tool in anthropometric research, including studies on the evolution of the pubic face with age.<sup>4,5,7</sup> However, studies discussing specific protocols for hardware and software configuration to allow for better inter-study comparisons are still very scarce. This study establishes the best practices and procedures for the Artec Space Spider and Artec Studio Professional software that produces the most accurate models for further analysis.

After establishing these protocols, this study tested the capabilities of Artec Studio Professional's Measure Tool and its use for age-at-death and sex estimation. The established protocols were used to produce models of the pubic symphyseal face. The surface area of these symphyseal faces were then calculated using the Measure Tool of Artec Studio Professional. No statistical significance was identified between the surface area of the pubic symphyseal face and age and sex of each individual. This suggests that another variable, such as perimeter or length of the face, may be useful to further investigate the morphological traits of the pubic symphyseal face. This presentation also discusses how a topological approach may be more useful than the geometric one to capture the type of variation encapsulated by the classic phase methods, not only to produce better age estimates, but also to infer and model the physiological processes underlying their age-related changes, including some of the main practical problems and challenges to capture and quantify that kind of information.

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### Biological Profile, 3D Models, Geometric Morphometrics



## A147 The Accuracy of Topographical and Shape Analysis Using 3D Surface Scanning Compared to Micro-Computed Tomography (Micro-XCT)

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**Learning Overview:** After attending this presentation, attendees will better understand the utilization of 3D surface scanning and micro-XCT modalities for population affinity estimation using topographical and geometric morphometric methods.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that 3D surface scanning can be utilized for population affinity estimation. Furthermore, 3D scanning modeling and advanced geometric morphometric analysis allow forensic anthropologists to analyze shape-related skeletal variation in a more accurate and repeatable way toward a more efficient biological profile.

The high number of unidentified bodies recorded in South Africa annually combined with substantial heterogeneity within the population necessitates that innovative forensic analysis methods be developed that incorporate variation from South African groups.<sup>1</sup> Although a sizeable 3D scanning modality exists in osteology and anthropology, developing new advanced methods and techniques involving 3D scanning for real-life forensic applications requires that the accessibility, practicality, and, more importantly, the accuracy of the methods be taken into consideration. To this end, this study aimed to assess the reliability of 3D surface scanning modality-based topographical and shape analysis comparison to gold standard micro-XCT reconstructions for estimating population affinity.

Crania of Black ( $n=22$ ) and White ( $n=22$ ) adult South Africans were obtained from the Pretoria Bone Collection (PBC), which is housed in the Department of Anatomy at the University of Pretoria, South Africa.<sup>2</sup> Two sets of 3D reconstructions were acquired from each cranium, namely micro-XCT scans and 3D surface scans. A total of 41 craniometric landmarks were manually placed on the 3D meshes using Amira-Avizo® 2019.3 software. 3D reconstructions and landmark datasets were acquired from both scanning modalities and compared using reproducibility testing, geometric discrepancies, and shape analysis. The landmark reproducibility testing demonstrated minimal dispersion errors for both landmark datasets, indicating that the placement of landmarks on 3D reconstructions from both modalities is reliable and repeatable. The results of the topographical analysis showed that micro-XCT and 3D surface scanning yielded similar 3D reconstructions. There were slight differences between surface models on the maxilla, the postero-lateral margin of the zygomatic and frontal process areas. The minimal geometric discrepancies observed between the reconstructions suggest that both modalities provide similar shape landmarks datasets and can be used without compromising the accuracy of the analyses.

The statistical findings showed similar population affinity-related mid-facial shape variations analyzed across both modalities using Geometric Morphometric Methods (GMM). The Principal Component Analysis (PCA) revealed similar landmark configuration clustering between the two modalities, emphasizing that the two-scanning procedure provided relatively similar shape data and similar shape analysis for population affinity-related shape variations.

The results obtained in this study suggest that 3D surface scanning can be utilized for population affinity estimation using GMM. Incorporating 3D scanning modalities and advanced geomorphometric analysis into the existing biological profile estimation allows for an accurate and repeatable analysis of shape-related skeletal variation. 3D imaging and scanning modalities are useful for forensic anthropology laboratories as the methodologies are non-invasive, ethical, easily storable, and retrievable. In South Africa, digital repositories of cranial data of unidentified persons are far more manageable for storage and analysis than physical skeletal material. Furthermore, 3D analyses also open the possibility for analyzing a more significant number of cases each year.

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### 3D Scanning Modalities, Population Affinity Estimation, Geometric Morphometric Methods (GMM)

## A148 Myositis Ossificans Circumscripta: Evidence Contributing to the Reconstruction of a Life History

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**Learning Overview:** Accurate recognition and description of myositis ossificans circumscripta (traumatica) in human skeletal remains can be informative for the reconstruction of an individual's life history. After attending this presentation, attendees will understand the diagnostic features, pathophysiology, and occurrence rate of Myositis Ossificans Circumscripta (MOC). Several medical examiner cases will be presented to highlight the importance of recognizing and reporting various manifestations of MOC.

**Impact Statement:** In the field of medicolegal death investigation, thorough injury documentation is necessary for accurate cause and manner of death classification as well as appropriate case adjudication. When correctly identified, MOC may provide evidence of a traumatic history and assist in reconstructing the decedent's life history. The forensic science community will benefit from having a more in-depth understanding of the pathophysiology, occurrence rate, and presentation of MOC.

MOC, one form of heterotopic ossification, is a condition in which bone tissue forms within skeletal muscle, primarily after an injury or surgical intervention. Most often it occurs in young adults and athletes following an injury, but has been reported in all ages, including children as young as one year. The injury can result from a single event such as a hit sustained while playing a contact sport like football, or from repetitive injury such as within the thighs of a horseback rider. Although rare, MOC can occur following a severe muscle strain.

Typically, the clinical presentation of MOC is muscle pain that persists longer than would be expected for a simple muscle strain or contusion. The developing lesion can cause mechanical irritation of the surrounding bursa, tendon, or joint, but also paresthesia, weakness, lymphedema, and venous thromboembolic disease when the lesion compresses nearby neurovascular structures. The pathophysiology is incompletely understood; but, it is likely due to the mistaken differentiation of mesenchymal stem cells into chondrocytes and osteoblasts in the inflammatory-rich environment. The formation of the bone is gradual—often taking several weeks to develop after the injury or surgery—and results in a mass that often can be palpated. In most cases, MOC is a self-limiting and self-resolving process and surgical intervention is not needed; however, in extreme cases, surgery may be warranted.

*In vivo*, the features of MOC can be easily recognized radiologically, but possible mimics include osteosarcoma, synovial sarcoma, and parosteal osteosarcoma. In gross examination of the dry bone, MOC lesions are frequently adjacent to the cortical surface as a separate mass (although the lesions may eventually fuse to the adjacent bone) and are easily differentiated from other osteolytic or osteoblastic lesions.

The occurrence of MOC can be informative for reconstructing the life history of an individual and several publications have associated it with inflicted injury. In two cases investigated by the District of Columbia Office of the Chief Medical Examiner and Harris County Institute of Forensic Sciences, MOC was linked to the physical treatment of the decedent. One case involved a 6-year-old child abuse victim with bilateral MOC in the region of the *vastus lateralis* in the thigh. A second case involved a 33-year-old female who was found to have MOC on several long bones. Reportedly, the woman had a history of psychotic episodes during which her family would employ restraints. In both cases, the recognition of the MOC was important to demonstrating the history of inflicted injury.

During this presentation, these cases and others will be reviewed in depth as well as the pathophysiology and occurrence rate of MOC. The attendees will learn how to recognize MOC and gain an understanding of the value of the pathological condition in the development of a skeletal profile.

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### Myositis Ossificans Circumscripta, Trauma, Skeletal Profile

## A149 The Effects of Gender Reassignment Surgery and Related Hormone Treatment on Morphological Sex Estimation in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will understand the current status of research into the effects of gender reassignment surgery and related transgender hormone therapy in relation to human identification from skeletal remains.

**Impact Statement:** This presentation will impact the forensic science community by drawing together and evaluating the available evidence from the United Kingdom and elsewhere on the impact, if any, of transgender hormone therapy and gender reassignment surgery on the skeleton and how these procedures may impact human identification from skeletal remains.

In biological anthropology, prior to the 1990s, research was structured around dichotomous biological sex.<sup>1</sup> The acknowledgement of gender identity emerged more prominently in the late 1990s and is now increasingly being considered as an important part of the human identification process in forensic anthropology. Some research in this area has been published in the United States, but no literature was found relating to the medical pathways in the United Kingdom, or to their potential effects on identification of skeletal remains.

The aim of this study is to review the effects of gender reassignment surgery and related transgender hormone therapy in relation to sex estimation methods and identification in forensic anthropology in the United Kingdom. It was hypothesized that gender confirmation surgeries could affect skeletal remains and therefore impact on traditional non-metric sex estimation methods. The use of traditional sex estimation methods may be exclusionary of transgender individuals, especially those who have undergone gender reassignment surgery due to the binary allocation of biological sex derived from scoring of continuously variable skeletal traits. It can be further hypothesized that transgender-related hormone therapies may also influence skeletal remains, particularly if treatment is commenced prior to puberty and the ensuing development of sexual dimorphism in the skeleton. Peer-reviewed literature from the United Kingdom and elsewhere suggests that adult hormone therapy may impact bone density, but not the morphological appearance, and should not, therefore, affect non-metric sex estimation from skeletal remains.<sup>2</sup> On the other hand, gender confirmation surgery has been found to leave impressions, particularly on the facial region of the cranium and the mandible since metal plates, screws, and other biocompatible materials are sometimes used in facial gender confirmation surgery.<sup>3</sup> Consequently, evidence of specific patterns of facial surgery may assist in confirming transgender identity in an individual, and serial numbers/manufacturer information from implants may further assist the identification process.<sup>4</sup> It is concluded that further investigation is needed into the effects of pre-pubertal hormone treatment and gender confirmation surgery on skeletal remains to determine the impacts on the accuracy of non-metric sex estimation methods and to ensure that transgender identities are not excluded.

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### Forensic Anthropology, Transgender Hormone Therapy, Gender Reassignment Surgery

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## A150 Trotter's Tibia Error in Historical Context

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the historical context and details behind Trotter's tibia measurement error.

**Impact Statement:** This presentation will impact the forensic science community by providing previously unpublished historical details on the source of this error, which continues to affect stature estimation.

Mildred Trotter made a now well-known error in the tibia measurement for her stature estimation publication in 1952.<sup>1</sup> This has had longstanding repercussions for anthropological research and forensic applications that rely on the formulas, and the error is frequently revisited in the literature. This presentation provides novel historical details on the conditions under which Trotter made the error and how it evaded scrutiny for decades before being exposed.

Trotter measured the tibia without the medial malleolus, but described the technique as including the malleolus for her 1952 publication. This presentation addresses the variety of contemporary tibia measurement standards and the multiple measurement techniques Trotter simultaneously used for United States Army World War II casualty identification casework and for research. Details are also provided on Trotter's survey of prominent anthropologists for their opinions on tibia measurement techniques (regarding not just how the bone is measured in practice, but how they thought it should be measured) when beginning her stature research, including responses from T. Dale Stewart and Wilton M. Krogman.

The general problem with these 1952 stature formulas using tibia measurements was first acknowledged by Trotter and her co-author, Goldine C. Gleser, in their 1958 paper, which expanded to add data from Korean War remains.<sup>2</sup> This presentation provides information on how they continued to miss the source of the tibia problem. To Trotter and Gleser, it simulated secular change, populational variation, or age-related proportional changes. It got lost in the extensive noise in the data, and they never sorted out the source of the error. The problem was also widely acknowledged in many other research publications (even as early as 1958) and this presentation addresses possible explanations for why such a clear issue did not attract much attention for decades. Once this systematic error was made, it was insidious, unlike the easily correctible errors uncovered for bones such as the radius and ulna that resulted from simple typos.

The source of the error was first identified by the landmark work of Richard Jantz, David Hunt, and Lee Meadows Jantz in 1994 and 1995.<sup>3, 4</sup> This presentation responds to suppositions made by Jantz et al., including questions of possible inexperience or intentional concealment, with details from Trotter's archival materials and relevant historical information.

The goal of this presentation is to provide further context for anthropologists using, modifying, and investigating this influential error.

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### Tibia, Mildred Trotter, Scientific Error

## A151 Age Estimation From Cranial Suture Obliteration: A Computed Tomography (CT) -Based Evaluation

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**Learning Overview:** Cranial suture undergo obliteration in a predictable manner as the age of the individual progresses. The extent of obliteration can aid the medicolegal professionals in estimating age. This study employs the use of Acsadi and Nemeskri scoring method on the ectocranial suture closure on 3D CT images for the purpose of age estimation. Both linear and multiple linear regression models were applied for the purpose of age estimation. Attendees will learn about the degree of cranial suture obliteration in 3D CT scans of the skull, as well as how the Acsadi and Nemeskri scoring method can be applied to the ectocranial surface instead of the usual endocranial surface.

**Impact Statement:** This study used the Acsadi and Nemeskri scoring method to estimate age based on cranial suture obliteration in 3D CT images. When additional skeletal age markers are missing, such as in the case of mutilated or dismembered remains, the approach can be used alone or in conjunction with other well-established age estimate methods.

Age estimation is a critical aspect of human identification. Most of the common methods of age estimation include assessment of epiphyseal fusion of the long bones and the assessment of dental maturity. Most of these indicators of an individual's age attain complete maturation by the age of 25 years, therefore, age is routinely estimated using cranial suture obliteration and morphological changes of the pubic symphysis for adults. As an individual ages, their cranial sutures undergo obliteration in a predictable manner.

The present study investigated 3D CT scans of 262 individuals (182 males and 80 females), ranging in age from 18–85 years, to assess the extent of ectocranial cranial suture closure. The Acsadi and Nemeskri scoring method was used to evaluate the ectocranial suture closure and obliteration from the 3D images. The 3D CT images were obtained from the Department of Diagnostic and Interventional Radiology, All India Institute of Medical Sciences (AIIMS), Jodhpur, from patients who underwent CT examination of the head for diagnostic purposes. Patients suffering from any skeletal/bony deformities/injuries and congenital, acquired, metabolic, nutritional, and hormonal defects were excluded from the study.

A statistically significant correlation was observed between the chronological age and cranial suture obliteration scores ( $P < 0.05$ ). Simple and multiple linear regression models were developed using the cranial suture obliteration scores to estimate age. For females, the simple linear regression model developed using the coronal suture obliteration scores provided the least Standard Error of the Estimate (SEE) (SEE=11.218 years), while in males, the regression model developed using lambdoid suture obliteration scores showed the least SEE (SEE=15.021 years). When the sex of the sample was not considered, the least SEE was observed for the sagittal suture (SEE=14.554 years). The SEEs for multiple linear regression models developed for estimating age using obliteration scores of sagittal, coronal, and lambdoid sutures were 14.808 years in males, 11.131 years in females, and 13.956 years in the total study population. The study concluded that cranial suture obliteration, as scored using the Acsadi and Nemeskri scoring method, can be used to estimate age from 3D CT images. The method can be employed when other indicators of the skeletal age are absent, as in cases of mutilated or dismembered remains, or in adjunct with other well-established methods of age estimation.

### Forensic Age Estimation, Cranial Suture Obliteration, Acsadi and Nemeskri Scoring Method

## A152 Age Estimation From Pubic Symphyseal Morphological Changes: A Computed Tomography (CT) -Based Evaluation

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**Learning Overview:** This study evaluates the utility of the Chen et al. method for age estimation by studying changes in the pubic symphysis using CT scan images.<sup>1</sup> It can be useful for forensic medicine and anthropology experts.

**Impact Statement:** In this study, the Chen et al. method, which is an autopsy study-based method for age estimation by studying changes in the pubic symphysis has been implemented using CT scan images in living individuals.

Age estimation is an essential component of the process of identification, both in living and deceased individuals. The pubic symphyseal surface consistently shows degenerative observational variations throughout the lifespan, making it a highly reliable feature for age estimation. The present study aims to establish an individual's age, based on the morphological changes observed on the pubic symphyseal surface, using criteria similar to the Chen et al. method using 3D reconstructed CT scan images of the pubic symphyseal surface.

The 3D CT images of the pelvis were obtained from the Department of Diagnostic and Interventional Radiology at the All India Institute of Medical Sciences in Jodhpur for this investigation. The study population consists of 154 male individuals who were 15 years and older. Morphological age indicators were assessed and scored following methods described by Chen et al. In the present study, both linear and multiple regression analysis were developed. It was concluded that the morphological changes occurring on the symphyseal surface were significantly correlated with the chronological age. Among all of the morphological indicators, bone density was the best predictor of chronological age, followed by the ventral rampart, ridges and furrows, and the lower extremity on the symphyseal surface. The ossific nodule was the least reliable parameter in estimation of age. The standard error of estimate of age in males was 7.19 years, which is relatively higher when compared to the study done by Chen et al., having a standard deviation of the age of 2.13 years. It is also observed that the standard error of estimation in the present study is relatively lower than that of the study done by Fleischman, having a standard deviation of 9.54 years.<sup>2</sup> Morphological features on the pubic symphyseal surface do not exhibit bilateral differences. From the present study, it was also concluded that the Chen et al. method is applicable to 3D CT images of the pubic symphyseal surface to estimate age.

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### Forensic Anthropology, Age Estimation, Pubic Symphysis

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## A153 Personal Identification of the L1-L5 Lumbar Vertebra Using Antemortem Computed Tomography (AMCT) and Postmortem Computed Tomography (PMCT) Scans

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**Learning Overview:** After attending this presentation, attendees will gain a deeper understanding of the usefulness of part-to-part comparison of anatomical features comparing AMCT and PMCT.

**Impact Statement:** This presentation will impact the forensic science community by providing the results of the initial findings of an ongoing study utilizing part-to-part comparison of the lumbar vertebra for personal identification. This presentation will examine the real-world application of using CT-derived 3D models of the L1-L5 vertebra of AMCT scans of known individuals and compare them against true PMCT scans to establish the validity of using this technology for personal identification.

The confirmation of identification for an unknown individual is a critical part of forensic practice especially in Disaster Victim Identification (DVI). The comparison of antemortem imaging for the purposes of personal identification is a common tool in pathology, odontology, and anthropology. A simulated version of this study was conducted successfully and served as a proof of concept of using 3D-rendered lumbar vertebra comparisons as a means for personal identification.<sup>1</sup> This study applies those findings to test true AMCT scans against true PMCT scans for the purposes of personal identification.

The University of Leicester, East Midlands Forensic Pathology Unit utilizes PMCT extensively in their daily practice and DVI situations. For the purposes of this project, Leicester acquired 23 matching AM scans for individuals that passed through their facility for PMCT scanning. The University of Leicester anonymized the scans so researchers at the University of South Florida Health Department of Radiology were blinded to the identities of the AM and PM scans. Each scan was imported into the Mimics® Innovation Suite v. 24. The L1-L5 vertebra were then isolated and modeled via segmentation and thresholding. Each series of 23 AM vertebra were registered with a target unknown PMCT-derived vertebra. A part-to-part comparison was conducted for each vertebra and a percent match was measured. A threshold of +/- 1mm was set for the part comparison.

Every unknown PMCT L1-L5 was correctly matched to the corresponding AMCT L1-L5, signifying complete accuracy for this sample. A Receiver Operator Curve (ROC) was calculated to determine 100% sensitivity and specificity with a cutoff point of a 0.735 match ratio. True identifications had an average match ratio of 0.945 +/- 0.048. Negative identifications had an average match ratio of 0.367 +/- 0.01.

With the increase usage of PMCT in the forensic sciences, there is an equal increase in the availability and opportunity to utilize 3D tools. This study has demonstrated the utility of 3D part-to-part comparison for successful personal identification.

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### Part-to-Part Comparison, Disaster Victim Identification, Forensic Radiology

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## A154 The Limitations of Sex Estimation: A Comparison of Osteological, Genomic, and Proteomic Methods

*Glendon Parker, PhD\*, University of California-Davis, Davis, CA; Jane Buikstra, PhD, Arizona State University, Tempe, AZ*

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**Learning Overview:** After attending this presentation, attendees will learn about molecular methods for skeletal sex estimation and their limitations.

**Impact Statement:** Skeletal sex estimation is critical in forensic anthropology, but the limitations of different anatomical and molecular methods are poorly delineated. The advent of proteomic sex estimation provides a counterpoint to establish osteological and genomic methods and allows the limitations of different approaches to be statistically developed.

Sex estimation of skeletons is fundamental to interpret and reconstruct many forensic contexts. Currently, three approaches are available to estimate sex: osteology, genomics, or proteomics, but little is known about the relative reliability of these methods in applied settings. In order to evaluate the forensic efficacy of respective methods this study evaluated degraded skeletal material in two settings, two ancestral Ohlone sites in Central California (2,440 and 100 cal BP,  $n=55$ ), and the Lower Illinois River Valley (Late Woodland to Archaic horizons,  $n=8$ ). Sex estimation was possible in 100% of both samples using proteomics, and the Californian context 91% using genomics, and in 51% using osteology. Agreement between the methods was high; however, conflicts did occur. Genomic sex estimates were 100% consistent with proteomic and osteological estimates when DNA reads were above 100,000 total sequences. However, more than half the samples had DNA read numbers below this threshold, producing high rates of conflict with osteological and proteomic data where 9 out of 20 conditional DNA sex estimates conflicted with proteomics, a rate close to random. No such pattern was clear when mapping conflicting sex estimates onto proteomic data where conflicting sex estimates occurred across different proteomic data quality levels. Together, these data imply that conflicting sex estimates are due to poor quality DNA and not proteomic data. This is supported by the finding that proteomic data quality is more stable compared to DNA. While the DNA signal decreased by an order of magnitude in these samples, there was no decrease in proteomic signal.

The study implies that sex estimates can be obtained from degraded material in forensic contexts. Osteological sex estimation is reliable (i.e., consistent with other techniques when sample signal is high), but has a high rate of indeterminate sex assignment when fragmentary and juvenile remains are assessed. Genomic methods help to extend sex estimation to many juvenile or fragmentary remains, but had a high rate of conflict with osteology or proteomic estimates for conditional sex assignments below the 100,000 mapped read threshold. In the event of a conflict in sex estimation, conditional DNA-based estimates should be disregarded in favor of osteological or proteomic methods. Proteomic sex estimation was the most sensitive technique, providing results in all remains tested for both Californian and Illinois contexts, due in part to the stability of the amelogenin peptide signal. These findings are contingent upon the preservation of dentition associated with each burial. To obtain the greatest coverage and confidence in sex estimates for degraded skeletal material, proteomic approaches should be combined with osteological and genomic methods.

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**Sex Estimation, Amelogenin, Enamel Proteome**



## A155 A Computed Tomography (CT) -Based Evaluation of the Acetabulum for Age Estimation in an Indian Population

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**Learning Overview:** The present study highlights the significance of age estimation via a scored evaluation of the Calce method. Regression equations generated from the assigned scores can be used to estimate age more accurately in cases of forensic significance.

**Impact Statement:** The acetabulum is particularly advantageous for age estimation owing to its durability, slow development into maturity, and prolonged degeneration associated with old age. Computed tomographic (CT) evaluation of age-progressive degenerative morphological changes enables easier and quicker analysis without having to delve into the time-consuming and destructive process of maceration. The Calce method simplifies the process of age estimation through the scrutiny of defined morphological features within the acetabulum. A scored evaluation of these morphological features laid down by Calce can be used to generate population-specific regression models to allow for a more accurate age estimation using the acetabulum.

Age estimation constitutes an important aspect of forensic research and investigation.<sup>1</sup> Within the skeletal framework, dental development and mineralization, ossification changes, and degenerative morphological changes of various bones have often been relied on for the purpose of age estimation.<sup>2,3</sup> Among these bones, age-progressive changes in the innominate bone are of particular importance when it comes to age estimation across varying age groups.<sup>4-6</sup> Within the pelvis, the acetabulum presents as a durable and well-preserved feature, and the manifestation of characteristic age-related changes can be employed for age estimation.<sup>7</sup> The present study analyzed CT scans in order to evaluate acetabular changes for the purpose of age estimation in an Indian population. CT images of 250 individuals aged 10-88 years were scrutinized according to the features defined under the Calce method of acetabular age estimation. Scores were allotted to the various features and a cumulative score was calculated. No significant bilateral and sex differences were observed. Significant correlation was obtained between the scores for these defined changes and the chronological age of individuals, and population-specific regression models were generated. Mean absolute error observed with the test sample was 9.24 years, with a mean difference of 0.45. Observations of the present study can be employed for age estimation from the acetabulum in cases of medicolegal significance.

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### Forensic Age Estimation, Computed Tomography (CT), Regression Models

## A156 Toward a Model for Estimating Postmortem Interval (PMI) at High Altitude: Categorical Macromorphoscopic Changes Observed Throughout the Trajectory of Human Decomposition in the Colorado Rocky Mountains

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**Learning Overview:** After attending this presentation, attendees will understand: (1) unique categorical changes observed along the continuum of human decomposition in a high-altitude environment, rendering the Total Body Score (TBS) model insufficient for use in this region; and (2) how this novel work provides the foundation for developing a region-specific predictive model for PMI estimation.

**Impact Statement:** This presentation will impact the forensic science community by addressing the environment-specific data gap pertaining to rate, pattern, and trajectory of decomposition in a high-altitude environment and supplement the growing body of knowledge pertaining to environment-specific patterns of decomposition.

Human decomposition is a dynamic process composed of several intercalated variables. The catalytic potential of individual and interacting variables exists along a continuum, rendering variable-interaction neither constant nor wholly predictable. Complexity is intensified in outdoor death scenes when the environment is not well understood from a holistic forensic-ecological perspective.

High-altitude environments are associated with interacting ecological, atmospheric, behavioral, and physiological variables. *In vivo* physiological adaptations include modified physiopathways to promote oxygen transport, hemoglobin production and concentration, nitric oxide synthesis, blood flow regulation, and increased respiration. Non-adaptive changes include higher-than-national-average incidence of suicide, exacerbation of pre-existing chronic conditions, and initiation of acute pathogenesis, including high-altitude cerebral and pulmonary edemas.<sup>1-2</sup> Summarily, variables present at high-altitude are hypothesized to affect the suite of biophysiological changes presented throughout decomposition.

Controlled studies of decomposition in high-altitude environments are limited. Baigent et al. concurrently placed a small sample of porcine carrion at high-altitude (2,800 meters Above Mean Sea Level [AMSL]) and lower-altitude (1,200 meters AMSL) sites in Colorado.<sup>3</sup> The TBS model was used to assess decomposition, and accumulated Degree Days (ADD) were used as indices of time and temperature.<sup>4</sup> Results demonstrated that inter-site rate, trajectory, and pattern of decomposition varied significantly. Further, the TBS model was not sufficient for estimation of PMI at either site. However, controlled observation of decomposition within a human cohort is lacking and constitutes a critical analytical gap in high-altitude medicolegal investigation.

This longitudinal study seeks to address the lack of data pertaining to rate, pattern, and trajectory of decomposition at high-altitude with the ultimate goal of: (1) creating a holistic region-specific taphonomic profile; and (2) developing predictive models for the estimation of PMI. This study is currently conducted at Colorado Mesa University's Forensic Investigation Research Station high-altitude satellite facility (FIRS-TB40). FIRS-TB40 is located in the Rocky Mountain Region of Colorado at 2,900 meters AMSL. The environment is characterized by slopes, alpine forests, moraine grasslands, rapid weather shifts, concentrated incidents of heavy precipitation, high Ultraviolet (UV) exposure, and heavy annual snowfall. Placement of human remains began in March 2020; to date, ten human donors have been placed, unclothed, supine, at surface level in 10'x10' steel cages situated on a 45° slope. An onsite weather station is deployed to collect hourly data points for 13 environmental variables, including temperature and precipitation. Donors are photographed and decomposition changes are recorded daily using the TBS model. While TBS has not performed well as a predictive model in this region, it was employed throughout the first year to establish a baseline against which to identify divergent variables, as well as facilitate testing of the model among a human cohort at high-altitude.

Seven categorical changes have been observed throughout the cohort. These include: (1) pattern and spectrum of color change; (2) adipocere formation with late-stage interdigitated saponification of dermal layers; (3) fluid bloat leading to premature skeletonization of the affected tissue; (4) gravity-induced slope roll; (5) pseudoburial as a result of adhesion and slope wash; (6) anterior/posterior differential decomposition; and (7) peripheral dermal drying and retraction resulting in islands of fresh tissue surrounded by desiccated dermis. Additionally, the TBS model was not sufficient for estimation of PMI due in part to the unique suite of categorical changes observed within the high-altitude human cohort. In this case, model mimicry could not be achieved. While typically an analytical hazard, the inability to achieve model mimicry in this study demonstrates the critical need for environment-specific models for PMI estimation.

Future study will test these categorical variables for significance among environmental variables, with the goal of establishing predictive models for PMI estimation.

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### High Altitude, Decomposition, Region-Specific

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## A157 Modeling Scavenging Patterns Within the Willamette Valley of Oregon to Inform Search Structure in Medicolegal Investigations

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**Learning Overview:** After attending this presentation, attendees will understand how animal scavenging within the Willamette Valley of Oregon compares to previous research done in this region and beyond; how scavenging activities influence the decomposition process; and how differing sub-environments and seasonality influence scavenging behavior.

**Impact Statement:** This presentation will impact the forensic science community by increasing the data available on animal scavenging in an understudied region.

Forensic taphonomic research has indicated non-human animal scavengers play an important role in the decomposition, dispersal, and bone destruction of human remains in an outdoor environment.<sup>1</sup> Understanding the scavenging patterns of various animal species in regard to human remains can help forensic scientists locate and recover dispersed skeletal elements, understand the difference between scavenger damage and antemortem/perimortem trauma, as well as better inform estimates of the Postmortem Interval (PMI).<sup>2</sup> Wescott states that there is a gap in the literature focused on the behavior of animal scavengers.<sup>3</sup> Understanding the intricacies of scavengers in a particular region allows professionals in the medicolegal field to more efficiently reconstruct the events occurring after death. Scavenging activities can vary depending on the region and climate due to differences in species presence, topography, seasonality, niche specificity, and available resources. Currently, there are few studies focusing on animal scavenging within West Coast states of the United States. Adding novel research to the body of literature will provide forensic investigators in the western United States more insight to accurately estimate PMI and construct a search structure that takes into account scavenging activity in medicolegal investigations.

In the current study, six whole hog (*sus scrofa*) carcasses were used as human analogs and placed on the ground surface in two separate outdoor environments (forested and open) in the summer of 2021 at the Multidisciplinary Oregon Taphonomy Investigative Studies (MORTIS) facility in Eugene, OR. Carcasses were purchased after ethical euthanasia via gunshot to the head and were animals destined for consumer use. The remains were distributed across an 80-acre forested parcel of land as well as a separate 4-acre disturbed meadow environment. Each environment had a control carcass that was caged in order to inhibit animal scavenging. The inclusion of a control carcass allowed researchers to record non-disturbed decomposition processes in order to analyze the effect that animal scavenging has on the pattern and timing of the decay process. Game cameras were utilized throughout the study to record scavenging behaviors and monitor dispersal of remains. Daily monitoring of the carcasses occurred from Day 0 to Day 21 of the observation period, followed by every-other-day observations from Day 22 to 33, and finally weekly observations from Day 34 to 60. Observational visits included scoring decomposition and noting any noticeable changes indicative of animal scavenging (i.e., disarticulation, dispersal, visible bite marks on soft tissue, mapping of dispersed remains, etc.).<sup>4-5</sup>

Observed scavengers included: turkey vultures (*Cathartes aura*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), striped skunk (*Mephitis mephitis*), spotted skunk (*Spilogale gracilis*), unspecified small rodent species, and various small bird species. This study found notable differences between open and forested environments in both scavenging and decomposition. This included skeletonization occurring in open scavenged remains within 48 hours of placement and an extended period of mummification in forested and control carcasses. Data from this study was analyzed in conjunction with previous research from the facility in 2019 and results are consistent in patterns of seasonality and scavenger diversity that can be utilized by medicolegal professionals when constructing search protocols.

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### Taphonomy, Animal Scavenging, Search Structure

## **A158 Forensic Taphonomy of Animal Scavenging in Europe**

*Lara Indra, MSc\*, Institute of Forensic Medicine Bern, University of Bern, Bern, Switzerland; Sandra Losch, PhD, Institute of Forensic Medicine Bern, University of Bern, Bern, Switzerland; David Errickson, PhD, Cranfield University, Cranfield, England, United Kingdom*

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**WITHDRAWN**

## 159 Testing the Efficacy of Bone Surface Swab Sampling of Microbial Colonization to Determine Postmortem Submersion Interval (PMSI) in Lentic and Lotic Environments

Sarah Rose, BSc\*, Virginia Commonwealth University, Richmond, VA; Hannah Johnson, MSc, Virginia Commonwealth University, Richmond, VA; Claire Cartozzo, PhD, Virginia Commonwealth University, Richmond, VA; Jenise Swali, PhD, Virginia Commonwealth University, Richmond, VA; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA

**Learning Overview:** This presentation will provide attendees with an understanding of microbial sequencing methods for estimating PMSI.

**Impact Statement:** This presentation will impact the forensic community by demonstrating that PMSI estimation of waterlogged skeletal remains can be achieved using surface swab sampling as an alternative to bone sampling.

In medicolegal death investigation, estimating the PMI contributes valuable information, and estimating the Postmortem Submersion Interval (PMSI) can specifically aid investigations involving victims discovered in aquatic environments.<sup>1</sup> Water-related deaths and homicide victim disposals often result in an increase in time required to discover the remains, causing loss of physical forensic evidence and potentially more advanced decomposition.

As demonstrated by Cartozzo et al., the succession of microbial communities found on skeletal porcine remains can be used to estimate the PMSI.<sup>2,3</sup> This study effectively used bacterial structure from bone tissue to develop bacterial succession models for prediction of PMSI.<sup>2,3</sup>

Though effective, direct bone sampling (bone powder) requires advanced processing techniques resulting in destruction of decedent tissue, this study investigates the use of bone surface swabbing as an effective alternative method to bone sampling that requires fewer technician hours, less sophisticated equipment, and simpler techniques for sampling.

Skeletal porcine (*Sus scrofa*) remains (rib and scapulae) were caged and submerged in both lentic (Henley Lake, Crozet, VA) and lotic (Rice Rivers Center, James River, VA) environments. In the Cartozzo et al. experiments, one cage, each containing five scapulae and five ribs, was pulled for sampling and water samples were collected every 250 Accumulated Degree Days (ADD).<sup>2,3</sup> In this study, swabs and water samples from the original experiment were analyzed at 500 ADD intervals, from baseline (0 ADD) to 4,500 ADD. DNA was extracted from bone surface swabs using the ChargeSwitch® gDNA Plant Kit and protocol, and variable region 4 (V4) of 16S rDNA was amplified and sequenced using Illumina® MiSeq® Sequencing platform. Sequence analysis was performed with Mothur (v.1.39.5) using the Mothur MiSeq® SOP and R (v.4.04).

For the Henley Lake samples ( $n=57$ ), Analysis of Molecular Variance (AMOVA) indicated a significant difference in beta diversity between the swab and bone samples ( $p<0.001$ ) and the rib and scapula samples ( $p<0.001$ ). These differences are likely explained by relatively greater Clostridia found in the bone samples compared to the swab samples, and by predominant Clostridia found in the rib samples compared to both Clostridia and Holophagae in the scapulae samples. ADD demonstrated a significant effect on alpha diversity ( $p<0.001$ ) with alpha diversity increasing with ADD. Random forest models to predict PMSI were constructed using swabs for both ribs ( $R^2=0.85$ , RMSE=582.33 vs.  $R^2=0.90$ , RMSE=522.91 in bone) as well as scapulae ( $R^2=0.85$ , RMSE=588.19 vs.  $R^2=0.96$ , RMSE=533.48 in bone).

Similarly, for the James River samples ( $n=32$ ), AMOVA indicated a significant difference in beta diversity between the swab and bone samples ( $p<0.001$ ) and the rib and scapula samples ( $p<0.001$ ). These differences are likely explained by predominant Clostridia and Gammaproteobacteria in the bone samples compared to the swabs and the overall variable presence and abundance of top taxa between rib and scapulae samples. ADD also demonstrated a significant effect on alpha diversity ( $p<0.001$ ), with alpha diversity increasing with ADD. Random forest models to predict PMSI were constructed using swabs for both ribs ( $R^2=0.74$ , RMSE=675.47 vs.  $R^2=0.94$ , RMSE=476.61 in bone) as well as scapulae ( $R^2=0.63$ , RMSE=783.07 vs.  $R^2=0.93$ , RMSE=500.7 in bone).

Swab samples from both aquatic environments predicted PMSI, albeit less accurately than bone powder, though this may well be due to the reduced sample of swabs relative to bone used in this study. These results suggest that bone surface swabbing provides an accurate, less labor-intensive, and non-destructive alternative for sampling skeletal remains to perform microbiome-based PMSI prediction.

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### Aquatic Taphonomy, Bacterial Microbiome, Submerged Bone Degradation

## A160 Improving Age-at-Death Estimation in Forensic Anthropology Applying a Translational Approach

*Sara C. Zapico, PhD, New Jersey Institute of Technology, Newark, NJ; Quentin Gauthier, PhD, Florida International University, Miami, FL; Aleksandra Antevska, BS, University of Tennessee, Knoxville, TN; Bruce McCord, PhD, Florida International University, Miami, FL*

**Learning Overview:** After attending this presentation, attendees will consider the possibility of applying knowledge acquired from biomedical research to improve age-at-death estimation in forensic anthropology.

**Impact Statement:** This presentation will impact the forensic science community by introducing a novel, innovative approach for age-at-death estimation, reaching accuracies of +1.7-2.05 years actual age.

As part of the biological profile, age estimation represents one of the key parameters toward the identification of human remains. This estimation is less accurate in adult individuals because it is based on degenerative changes in bones and teeth, being affected by endogenous and exogenous factors, pathological conditions, and fragmentary remains.

The application of biochemical techniques, based on the natural process of aging, may provide a potential solution to this problem. One of the current trends in aging research is epigenetics, the study of heritable changes in gene expression. Epigenetic DNA methylation, which involves the addition of a methyl group to a cytosine nucleotide in C/G regions, known as CpG islands, has led to the creation of “epigenetic clocks,” which are based on the correlation between methylation patterns and age. As a result of these studies, several authors have tried to accurately determine age from biological fluids (blood, saliva, semen), to apply to criminalistics. There have been scarce studies performed for forensic anthropological purposes. The latest methods have used the whole tooth for this analysis; however, DNA methylation is tissue-specific, and it is important to independently analyze the various types of tooth tissue in order to correctly apply the technique.

Teeth are the hardest structures in the human body, and they can survive after everything else has decomposed. The dental pulp is located in the central region of the tooth and protected from external insults by hard tissues. It is formed by a stromal tissue containing nerves, blood, and lymphatic vessels. This unique location and structure make it the preferred substrate for DNA analysis.

Based on these premises, the aim of this study was to analyze methylation patterns in pulp tissue from adult individuals and correlate these patterns with age, improving current age-at-death estimates.

Twenty healthy-erupted third molars, ages between 22 and 70, were used in this study. Pulp was isolated from the rest of the tissues. After DNA extraction, 200ng of pulp genomic DNA was bisulfite converted and later amplified by Polymerase Chain Reaction (PCR) for the following genes: ELOVL2, KLF14, SCGN, NPTX2, and FHL2. Pyrosequencing was performed to analyze DNA methylation levels of the CpG sites of these genes.

Six ELOVL2 CpG sites were found significantly correlated with age. In contrast, only one KLF14 CpG site, one NPTX2 CpG site, and two CpG sites of SCGN were found significantly correlated with age. None of the identified CpG sites of FHL2 were significantly correlated with age, although the correlation coefficients were high.

A multiple linear regression analysis was performed, and four models were developed for age estimation based on the assessment of these five genes. After leave-one-out cross-validation, models 2 and 3 produced the best accuracy, with Mean Absolute Error (MAE) between estimated and chronological age of +1.7 and +2.05, respectively ( $p$ -value <0.001). Additionally, Pearson correlations were carried out to compare predicted and chronological ages, obtaining  $r=0.98$  in both models.

This research is the first to explore age-associated methylation in pulp tissue applying pyrosequencing and obtains higher accuracies than current methodologies. The findings from this study provide a new quantitative tool for estimating age at death, which, in combination with traditional age markers, should improve identification accuracy in forensic cases. Future research may be able to expand on these results, identifying new markers, using different types of teeth, and extending the age range.

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### Age-at-Death, Forensic Anthropology, DNA Methylation

## A161 Reference and Target Sample Age Distribution Effects in Juvenile Dental Age Estimation

Valerie Sgheiza, MA, MS\*, University of Illinois at Urbana-Champaign, Urbana, IL; Helen Liversidge, PhD, Queen Mary College of London, London, England, United Kingdom

**Learning Overview:** After attending this presentation, attendees will have learned about the effects of age distribution shapes and model types on point estimates of age. This presentation will provide an example procedure for applying machine learning theory to methods for estimating the biological profile and demonstrate the effect of model selection bias on error rates.

**Impact Statement:** This presentation will impact the forensic science community by providing steps for both reducing bias in age estimation methods and calculating representative error rates that will facilitate comparing methods in the literature.

There are many reasons why age estimation methods may perform differently. In order to understand the effects of individual factors such as population, age distribution, model type, or scoring system, it is important to vary each factor individually while holding the rest constant. In the specific context of juvenile dental age estimation, a single dataset and machine learning approach was used to systematically test three potential sources of difference: the age distribution of the reference sample, the age distribution of the target sample, and Bayesian vs. classical regression modeling. The hypothesis tested was that a Bayesian model with a uniform reference and target sample would provide the least biased estimates of age. This presentation is also a case study of the effects of model selection bias. Model selection bias occurs when error rates are used to select a best model from several options. Model selection attributes error entirely to the model when error is partially incidental to the data. Follow-up testing using new data may therefore produce larger errors than initially reported.

The age distributions considered were uniform, unimodal ( $\sim\text{Chi}^2(10)$ ), and U-shaped ( $\sim\text{Beta}(0.95,0.9)$ ). The models used were Bayesian Cumulative Probit (BCP) and Multivariate Adaptive Regression Splines (MARS). The initial dataset consisted of Moorrees et al. scores of left mandibular permanent teeth from 1,926 London children 6-19 years old.<sup>1</sup> One hundred replicates were performed for each of the 18 reference-target-model combinations with a reference sample size of  $n=320$  and a target sample size of  $n=80$ . Since reference and target samples had varying shapes, true cross-validation was not possible. Instead, for each replicate, the reference and target sample were drawn randomly according to their corresponding distributional weights, then individuals duplicated in the reference sample were replaced in the target sample by randomly drawing individuals of the same sex and year of age from the larger dataset. True and estimated age in the target sample were compared for bias and overall error using loess smoothing of the residuals and Root-Mean-Square Error (RMSE).

BCP and MARS showed different patterns of age-specific bias, while reference sample distribution affected the magnitude of bias. BCP produced upward bias in the mid-teens, likely due to few age-informative stages at this time. MARS showed downward bias near 12 years depending on reference distribution, with the least from a uniform reference. RMSE was lowest for BCP models with a uniform reference (mean RMSE of three target distributions=1.0896 years) followed by bimodal reference (mean=1.0985) and unimodal reference (mean=1.1781). RMSE for MARS models was consistently an order of magnitude larger with a nine-model average RMSE of 12.6566. Target sample distribution had no consistent effect. True test error of the best model (BCP and uniform reference sample) was calculated using a holdout sample of 52 individuals. This RMSE of 1.2962 was larger than all nine initial BCP model RMSE values with the exception of unimodal reference with unimodal target (RMSE=1.3518).

In conclusion, a uniform reference sample and Bayesian model provided the lowest error in point estimates of age, but true test error for this model was larger than error rates found during model selection, demonstrating potential model selection bias.

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### Dental Age Estimation, Method Optimization, Machine Learning

## A162 An Age-at-Death Estimation Using Cementochronology in Thermally Altered Teeth

Ryen Weaver, MS\*, George Mason University, Fairfax, VA; Anthony Falsetti, PhD, George Mason University, Fairfax, VA; Timothy Gocha, PhD, Texas State University, San Marcos, TX; Joseph DiZinno, DDS, George Mason University, Fairfax, VA

**Learning Overview:** After attending this presentation, attendees will understand the impacts of thermal alterations on the ability to estimate age at death through cementochronology in human teeth.

**Impact Statement:** This presentation will impact the forensic science community by testing the validity of using cementochronology to estimate age accurately and objectively in unknown individuals whose remains have been thermally altered.

Macroscopic age-at-death estimations provide age ranges that give broad and often insufficient insight to an individual's chronological age. Accurate age estimations can become more complex for a forensic anthropologist if the unknown individual has been subjected to extreme heat from an assortment of scenarios that include but are not limited to structure fires, airplane crashes, automobile accidents, and attempts to conceal evidence of homicide. Due to their high mineral content and sequestered placement in the jaw, teeth have the ability to be one of the best-preserved human tissue remains in extreme heat situations.<sup>1</sup> Cementochronology utilizes the cementum, the mineralized covering of a tooth root, as an aid in estimating an individual's age.<sup>2</sup> The utilization of cementochronology is one of the most accurate ways to estimate age at death due to the countable cementum annulations found in the cross-sections of the tooth root, directly correlating to the individual's age.<sup>3</sup>

This study aims to provide an in-depth analysis of thermal alteration to human teeth by various accelerants when utilizing the cementochronology method to build a biological profile. The sample in this study consisted of 36 teeth from both male and female donors from all odontological positions, excluding third molars, ranging in age from 9-87 years. Three accelerants (acetone, lighter fuel, and 87 grade gasoline) with varied volumes were used to determine if the readability of cementum annulations can remain accurate after alteration. The 36 samples were separated into groups of three to represent one accelerant and were further separated into groups of four. Each accelerant had four groups of teeth, totaling 12 teeth per accelerant and 3 teeth per assigned volume. Results indicated that the type of tooth had significant impact on the ability to count annulations, with single-rooted teeth yielding better visualization of cementum annulations to that of multi-rooted teeth. Annulations were able to be read and estimated after alteration with all three accelerants used in this study. However, acetone yielded the lowest results with the most severe alterations.

A novel three-step formula was developed to help approximate the amount of cementum annulations found within each sample. This formula involves taking the width of the total area of cementum (X) and dividing it by the width of three sets of clear, dark, and light annulations (Y) to come to the total number of incremental lines (n). This value of "n" is then multiplied by "Y" to come to an estimated age (E). Once the "E" value is found, the estimated age of eruption for that particular tooth type is added to E to come to an accurate estimated age at death. A Pearson Correlation Coefficient test found this formula to yield estimated tooth cementum annulations that were highly correlated with the actual ages of the individuals from whom the samples were collected from  $r=0.965$ ,  $p<0.001$ . After thermal alteration of teeth with accelerants, it was found that cementochronology remains an accurate and helpful tool for estimating age at death in unknown individuals.

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### Cementochronology, Thermal Alteration, Age Estimation



## A163 Estimating Adult Age Using Radiographs of the Proximal Femur

*Jong In Kim, HBSc\*, University of Toronto at Mississauga, Mississauga, Ontario, Canada; Tracy Rogers, PhD, University of Toronto at Mississauga, Mississauga, Ontario, Canada*

**Learning Overview:** After attending this presentation, attendees will have learned the viability of estimating an unknown adult's age by assessing bone involution of the proximal femur.

**Impact Statement:** This presentation will impact the forensic science community by evaluating and improving a technique for skeletal age-at-death estimation.

In 2012, Pryce tested the reliability of the traits and variants associated with involution of the proximal femur that have been used to estimate physiological age. She examined 125 radiographs housed in the University of Toronto at Mississauga (UTM) radiograph collection and determined that the established variants could not accurately be used independently, or in conjunction, to estimate an individual's age.<sup>1</sup>

The purpose of this research is to improve Pryce's age estimation method and to determine whether a Bayesian method is a more appropriate statistical approach to estimate age using bone involution of the proximal femur.<sup>1</sup> Pryce's method utilized five traits that had an accuracy rate of at least 80% and a precision of less than 10% error, criteria recommended by Rogers.<sup>1,2</sup> The traits include: the density and thickness of the cortical bone; the density of the first trabecular structure; the density of the secondary trabecular structure; the location of the apex of the medullary cavity; and the degree of cavitation.<sup>1</sup> She also reduced the number of trait variants to four or five.<sup>1</sup> The final age estimation is calculated by averaging the age ranges of each variant, which was determined from the mean value plus one standard deviation.<sup>1</sup> The method was tested with 50 radiographs, which revealed an accuracy rate of 100% and an intra-observer error rate of less than 6%.<sup>1</sup>

The current research tested Pryce's method using two different groups of radiographs: 29 were previously assessed by Pryce; and 50 were new images not included in Pryce's study.<sup>1</sup> All statistical analyses were performed using R (v. 4.1.0) software.<sup>3</sup> The inter-observer error of Pryce's original criteria was calculated by using the first group of radiographs. A weighted kappa (K) revealed a minimal inter-observer agreement for the cavitation trait and a weak agreement for the remaining traits. All traits had a  $p$ -value $<0.05$ . Intra-class Correlation Coefficients (ICC) showed that the medullary cavity has good reliability, while the remaining traits had moderate reliability. All traits had a  $p$ -value $<0.05$ , indicating that the agreement between observers was not due to chance or bias. The Wilcoxon signed-rank test with continuity correction revealed that the distribution between the observers is the same for all traits ( $p$ -value $>0.05$ ).

Pryce's method was modified to improve the inter-observer error by combining several variants to create a 5-traits, 14-variants approach. By decreasing the number of variants, the inter-observer error decreased; improvements were seen in the weighted K for every trait (ranging from 0.54 to 0.76), as well as the ICC for every trait except for the secondary trabecular structure (ranging between 0.68 and 0.83). The Wilcoxon signed-rank test ( $p>0.05$ ) indicates there are no differences between the two sets of data for all traits, except for the cortical bone ( $p=0.01073$ ).

The success of utilizing Bayesian statistics to predict age will be evaluated using the data from group one radiographs to establish the posterior probabilities, and the second set of data will be used to test the Bayesian approach.<sup>1</sup>

As the femur is one of the most recoverable skeletal elements, it is important for forensic anthropologists to develop techniques of age-at-death estimation specific to the femur.<sup>1,4,5</sup>

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### Forensic Anthropology, Age Estimation, Proximal Femur

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## A164 From Subadult to Adult: Growth of the Cranial Complex and its Implications in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will better understand the ontogenetic patterns of the cranial complex and be informed of the potential of craniometric techniques for estimating parameters of the subadult biological profile.

**Impact Statement:** This presentation will impact the forensic sciences community by thoroughly exploring ontogenetic cranial growth and identifying when the cranium can be confidently employed in biological profile methods.

Measurement of the human cranial complex is common in forensic anthropology to estimate parameters of the adult biological profile. However, it is unknown at what age the subadult cranium displays comparable levels of variation to the adult cranium. The purpose of this presentation is to bridge this gap by analyzing the patterns of ontogenetic variation in the subadult cranium. This presentation will address: (1) how variation of the cranial complex shifts from birth to adulthood, and (2) how subadult cranial variation compares to adult cranial variation. By doing so, this research is foundational for assessing the potential of craniometric data to improve subadult biological profile methods.

Thirty cranial Interlandmark Distances (ILDs) established from 36 craniofacial landmarks were collected on 3D virtual renditions of crania obtained from Computed Tomography (CT) scans of 571 subadults aged between birth and 20 years and of known biological sex from the Subadult Virtual Anthropology Database (SVAD). Individuals were assigned life history stage groups according to their age: infancy (birth to 2.99 years;  $n=102$ ), childhood (3 to 6.99 years;  $n=5$ ), juvenile (females: 7 to 11.99 years; males: 7 to 12.99;  $n=66$ ), adolescence (females: 12 to 17.99; males: 13 to 17.99 years;  $n=170$ ), and adulthood ( $>18$  years;  $n=178$ ). An adult sample ( $n=1,035$ ) from the Forensic Anthropology Databank (FDB) was included for comparison. Linear Discriminant Analyses (LDA) were used to explore the variation between each life history stage and biological sex. Next, the data was split into training (75%) and testing (25%) sets and LDA with repeated 10-fold cross validation were run to determine if: (1) the subadult cranium could be used to estimate life history stage and/or biological sex, and (2) what subadult life history stage is most similar to adults in terms of classification accuracy and within-group (co)variation.

The LDA results demonstrate a clear ontogenetic trajectory in cranial variation. CV1 explains variation between each life history stage (89%), while CV2 explains variation between biological sex within each life history stage (4%). The overall classification rate using five life history stages is 65% for the pooled-sex sample. When looking at each life history stage separately, there is some overlap in cranial size variation between adjacent groups throughout ontogeny, with classification accuracy decreasing from 97% in infancy to 68% in adulthood, with almost superimposed ranges of variation in adolescence and adulthood. FDB adults share similar mean CV1 scores and ranges of variation to the SVAD adolescents and adults, while CV2 mainly captures population differences. Sex estimation is the least accurate during infancy (64%), but the most accurate in the SVAD adolescent and adult samples and in FDB adults with rates of 83%, 90%, and 93% respectively.

Final adult size is reached by adolescence as is the appearance of significant cranial sexual dimorphism and the expression of population affinity. The FDB adults share similar patterns of variation and classification accuracies with the SVAD adolescents and adults. Stabilization of the multivariate distribution of the cranial complex in adolescence indicates the cranium could accurately estimate features of the biological profile, such as population affinity and sex, for individuals older than 12 (F)/13 (M) years of age.

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### Interlandmark Distances, Ontogeny, Classification

## A165 From Subadult to Adult: The Expression of Cranial Macromorphoscopic Traits Through Ontogeny

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**Learning Overview:** After attending this presentation, attendees will understand the ontogenetic patterns of cranial Macromorphoscopic (MMS) traits and the variation of trait expression according to age and population affiliation in subadult individuals.

**Impact Statement:** The results of this study will impact the forensic science community by providing knowledge on MMS traits in general and specifically the ontogenetic patterns of these traits, their frequency of expression according to age and population affiliation, and in particular the age at which they stabilize and are analogous to adult frequencies and could be employed in biological profile methods.

Cranial MMS traits are often used in forensic anthropology to study biological distance and estimate population affiliation as part of the biological profile for adult decedents. However, very little is known of their ontogenetic trajectories. Indeed, the current discussion over the use, or misuse, of these traits in forensic anthropology would benefit from additional knowledge regarding their patterns of expression throughout ontogeny. The aim of this study is to evaluate the growth trajectory of 13 cranial MMS traits commonly used in forensic anthropology.

Thirteen MMS traits were scored on the virtually reconstructed crania from Computed Tomography (CT) scans of 968 subadults aged between birth and 22 years from the Subadult Virtual Anthropology Database (SVAD). Scoring followed Hefner and Linde; definitions were slightly modified to fit virtual material.<sup>1</sup> To reveal the growth trajectory of each MMS trait, score frequencies were calculated according to population affiliation (American Black, American White, Hispanic, Native American) and for each Life History Stage (LHS) defined as: Infancy from birth to 2.99 years (N=294), Childhood from 3.00 to 7.99 years (N=89), Juvenile from 8.00 to 11.99 years for females (N=36) and 12.99 years for males (N=59), Adolescence from 12.00 to 17.99 years for females (N=92) and 13.00 to 17.99 years for males (N=160), Adulthood from 18+ years (N=238). Frequencies were compared across LHS to assess when the stabilization of trait expression occurred, which was achieved when the distributions were comparable to the adult sample in each population affiliation group.

The pattern of frequencies for MMS trait scores related to the orbital region, namely Inter-Orbital Breadth (IOB) and Orbit Shape (OBS), stabilize during Childhood. Three MMS traits relative to the nasal aperture stabilize during the Juvenile period: Anterior Nasal Spine (ANS), Nasal Aperture Width (NAW), and Nasal Aperture Shape (NAS). Traits relative to shape (INA, NBC, NBS, NO, PS, PBD) and size (PZT, MT) showed later stabilization during Adolescence.

There was a remarkable stability of all MMS traits by adolescence (12+ years) as well as clear patterns according to craniofacial regions. By exposing the developmental trajectories and patterns of MMS traits, anthropologists can be more informed regarding the continuum of phenotypic variation and can better understand the contribution of MMS traits to the biological profile in subadults.

*This research was funded by the NIJ Award 2019-DU-BX-0039.*

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### Phenotypic Variation, Growth and Development, Population Affinity

## A166 From Subadult to Adult: Understanding Univariate Craniometric Growth Patterns

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**Learning Overview:** After attending this presentation, attendees will better understand the timing of ontogenetic shifts in cranial dimensions from birth to adulthood through an examination of univariate craniometric growth patterns by sex, regions, and life history stages.

**Impact Statement:** This presentation will impact the forensic science community by documenting the age at which males and females reach craniometric maturity for each Interlandmark Distance (ILD) and within regions, as well as providing measurable growth trajectories that can be broadly applied to forensic casework.

Although the general development of cranial regions is well-documented from clinical and evolutionary perspectives, quantifiable examinations of subadult cranial growth trajectories across the entire ontogenetic period (from birth to adulthood) are still needed within biological and forensic anthropology. This research investigates the timing of cranial growth and development for individual variables and their associated regions and assesses when cranial maturity in size is reached. By better understanding the complex univariate growth patterns of the cranium, anthropologists can better estimate the developmental timing of cranial pathologies or developmental defects and improve parameters of the subadult biological profile.

A sample of 571 male and female subadults aged between birth and 20 years was used from the Subadult Virtual Anthropology Database (SVAD). Twenty-six ILDs were derived from 36 3D cranial landmarks collected from virtual renditions of the cranium reconstructed from Computed Tomography (CT) scans.<sup>1,2</sup> Results are discussed through the perspective of ontogenetic Life History Stages (LHSs) and three cranial regions: neurocranium, basicranium, and splanchnocranium. The LHSs are infancy (0–2.99 years; females=46, males=56), childhood (3.00–6.99 years, females=28, males=27), juvenile (7.00–12.99 years; females=29, males=41), adolescent (13.00–17.99 years; females=61, males=105), and adulthood (18.00+; females=78, males=100). Multivariate Adaptive Regression Splines (MARS) with ten-fold cross-validation were employed to capture the non-linear ontogenetic trajectory of each ILD and sexes were analyzed separately to account for differential timing in growth trajectories.

The majority of neurocranial measurements peak in size between 3–5 years (early childhood) and stabilize (i.e., plateau) thereafter regardless of sex. Some basicranial measurements (e.g., foramen magnum length, foramen magnum breadth) mimic the neurocranium with stabilization at a young age, while others (e.g., basion-nasion length, biauricular breadth) exhibit a secondary peak in size for the juvenile and/or adolescent LHSs. Splanchnocranium measurements exhibit the most complex, dynamic, and sexually dimorphic growth trajectories with measurements consistently increasing in size throughout ontogeny and stabilizing in late adolescence. For most measurements, males exhibit changes in growth trajectories between 4–5 years and again between 13–17 years. In contrast, females exhibit changes in growth trajectories between 7–13 years.

Males and females have comparable timing in growth trajectories for all three cranial regions prior to adolescence, illustrating the ontogenetic stability of early cranial growth. However, trajectories diverge based on sex during adolescence, reflecting well-known sex differences in the onset and duration of puberty. Overall, the variable and regional patterns observed here demonstrate earlier achievement of adult size for the neurocranium and basicranium when compared to the splanchnocranium. In understanding the growth trajectories and timing of stabilization for individual craniometric variables, forensic anthropologists can better mobilize these variables when estimating components of the subadult biological profile.

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### Ontogeny, Interlandmark Distances, Growth Trajectory

## A167 Are Stable Isotopes Truly Stable Enough for Forensic Applications? Forensic Stable Isotope Research at a Human Decomposition Facility

Melanie Beasley PhD\*, Purdue University, West Lafayette, IN

**Learning Overview:** After attending this presentation, attendees will have a better understanding of current forensic stable isotope research studies conducted at the Anthropology Research Facility (ARF), a human decomposition facility at the University of Tennessee, Knoxville, to address questions of postmortem alteration of stable isotope values in biological tissues.

**Impact Statement:** This presentation will impact the forensic science community by presenting results from forensic stable isotope studies at a human decomposition facility that provide unique research opportunities for innovative method development and validation of *in vivo* stable isotope values from biological tissues critical for region-of-origin applications of unidentified human remains.

Forensic stable isotope analysis is most beneficial to forensic investigations of unknown human remains in cases where standard methods of identification are unsuccessful. Stable isotope analysis capitalizes on the use of stoichiometric models to understand how elements on the landscape move throughout an entire ecological system, creating unique “fingerprints” of elements incorporated into human biological tissues.

Despite successful applications in forensic anthropology of stable isotope analysis, there are still basic research questions about alteration of biological samples after death and the offset between tissue types that needs to be addressed for wider adoption by the medicolegal community. The Forensic Anthropology Center’s (FAC) documented donors provide a unique opportunity for such research because of the biological tissues curated, varied scenarios for decomposition (buried vs. surface), and standardized documentation of life history movement.

The four tissue types (i.e., hair, fingernails, bone, and teeth) regularly curated by the FAC allows for basic geochemistry research on donors with known residential history. This is key for understanding how element signatures are incorporated and fractionate (i.e., offset in stable isotope values) between different tissue types with differential growth history. Additionally, as donors are placed in different scenarios at ARF, this allows for a wealth of knowledge to be gained about the diagenesis (i.e., alteration to element signatures due to post-depositional environment) of different stable isotopes in different tissues.

Current stable isotope research at the FAC surround research questions about the postmortem alteration of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  in hair and fingernails. This is of forensic importance because diagenesis of elemental signatures recorded in physical evidence may impact interpretations of region-of-origin predictions if isotope ratios are altered by the burial environment. Systematic alteration to stable isotope values can also be used in new innovative methods for determining Postmortem Interval (PMI). One research project at the ARF presented at the 2019 and 2020 AAFS annual meetings, investigated the use of systematic change in  $\delta^{15}\text{N}$  values of decomposing muscle tissue and maggots feeding on decomposing tissue to test applications of diagenesis for determining PMI in recently deceased individuals.<sup>1,2</sup>

Beyond questions of stable isotope diagenesis and fractionation research, the long-term curatorial efforts of the FAC serve as an ideal opportunity to investigate concerns in the field of forensic stable isotope geochemistry about the long-term storage impact to stable isotope values of tissue samples. One such study has been initiated on fingernails stored over two years in different scenarios to test the alteration in five isotope systems.

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### Stable Isotopes, Human Decomposition, Forensic Anthropology

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## A168 The Significance of Sociocultural Covariates in Stable Oxygen Isotope Analysis: Implications for Region-of-Origin Predictions for Global Populations

*Taylor Lambrigger, BS\*, California State University-Chico, Chico, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the significant role that sociocultural covariates such as poverty, indigeneity, and rurality play in local access to drinking water in Oaxaca, Mexico, and consequently how these factors influence the oxygen isotopic compositions of human bodily tissues used in forensic provenancing studies.

**Impact Statement:** As the forensic science community continues to develop methods in the United States and apply those methods to communities throughout the globe, it is increasingly important to consider how the lived realities for these different communities impact the assumptions used in stable isotope methods. The evaluation of isoscapes predictions for oxygen isotopes in Oaxaca provides an example of the need to investigate several assumptions that may affect region of origin predictions.

Stable isotope analysis involves a set of biogeochemical methods that have developed significantly over the past two decades. Specifically, forensic anthropologists and isotope experts have focused on oxygen isotopes because of its ability to help estimate probable regions of origin of unidentified individuals using tissues such as hair, nails, bones, and teeth. Prior research on this isotopic system in human populations has found that drinking water is geographically patterned and that tap water is often a reliable proxy for drinking water in assessing these geographical links between human tissues and geography. Though largely developed in the United States and areas of Europe, forensic anthropologists have begun to apply the method in a variety of global contexts, including assisting in identifying missing migrants at the United States-Mexico Border.

The focus of this presentation is to critically assess the assumption made that tap water is a reliable proxy for drinking water in oxygen isotopic systems as they are applied to global communities. Specifically, the development of isoscapes as tools to assist anthropologists in estimating region of origin rely on this assumption, which may not be appropriate for communities that do not have access to reliable or clean drinking water. However, little research has been done to date to explicitly test this assumption. The source of drinking water from 56 municipalities throughout the state of Oaxaca, Mexico, were recorded and compared with census data on indigeneity, rurality, and poverty. These census data indicate significant variation in the demographic makeups of the sampled municipalities, with six different categories of drinking water recorded throughout the sampled areas.

This wide range in drinking water sources indicates that further investigation into the isotopic systems of diverse global populations is necessary. Over 40% of the sampled municipalities rely on bottled water. The five most rural municipalities relied on stream water exclusively for drinking and cooking. One municipality relied exclusively on rain water during the wet season. Categorically, tap water is not an adequate representation of the sources of water consumed by local Oaxacans. The need to investigate if this conclusion extends isotopically is urgent. If the remains we seek to help identify are coming from various socioculturally diverse communities, we run the risk of missing these populations because of our assumptions.

Ultimately while the growth and development of isotopic methods, namely isoscapes, has been tremendous, it is imperative that anthropologists continue to question the assumptions made in our methods as we apply them to global communities. While recent isoscapes have mapped regions beyond the United States, these maps still largely rely on tap water data. A plethora of ethnographic evidence shows that sociocultural covariates have a direct impact on water insecurity in communities around the globe, and these data show that the sources of drinking water are variable across a single state in Mexico. Recognizing the varying sociocultural realities of the communities we seek to aid and identify is paramount as we grow and develop our methods moving forward.

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**Stable Isotope Forensics, Provenancing, Forensic Anthropology**

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## A169 The Detection of Bone Particles in Non-Carbonate Soils

*Angi Christensen, PhD\*, FBI Laboratory, Quantico, VA; Ian Saginor, PhD, FBI Laboratory, Quantico, VA*

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**Learning Overview:** Attendees of this presentation will learn a new technique for determining whether a non-carbonate soil may contain bone particles.

**Impact Statement:** The forensic science community will be aware of a new, simple, cost-effective method for determining whether a non-carbonate soil may contain small particles of bone.

For many years, geologists have used a simple test to determine whether a soil contains carbonate minerals such as calcite and dolomite. This test involves the application of a weak Hydrochloric Acid (HCl) and observation for an effervescence reaction, the intensity of which can be graded on a qualitative scale.

This routine soil test was used in a recent forensic geology investigation. Based on the geographic recovery location, the soil was not expected to contain carbonates, but expressed effervescence in the presence of HCl. The soil was recovered from a location known to be in close proximity to human remains, and a collaborative assessment of the soil by a forensic geologist and forensic anthropologist confirmed the presence of small bone fragments in the soil that were visible microscopically. The question was then posed whether small, degraded bone particles in the soil could be causing the effervescent reaction rather than carbonates. This study therefore tested whether non-carbonate soils exhibit an effervescent reaction in the presence of HCl if bone particles are present in the soil.

A soil sample was collected from Quantico, VA, and tested for pre-existing carbonates. X-Ray Diffraction (XRD) failed to detect carbonate minerals, and the soil showed no effervescence in the presence of HCl solution. A portion of the soil was lightly ground with a mortar and pestle to break up the soil aggregates and to facilitate mixing. Five non-human bone samples were utilized, representing a variety of taphonomic alterations, including weathering, as well as one bone that was burned. The bones were ground using a ceramic mortar and pestle and sieved to achieve particle size fractions of <0.50mm and 0.50–0.85mm. A sample of powdered National Institute of Standards and Technology (NIST) hydroxyapatite standard was also used.

For each bone particle fraction as well as the hydroxyapatite standard, a 10:1 mixture by weight of soil:bone was prepared. A small sample of each mixture was placed into a crucible and positioned under a stereobinocular microscope at ~6.3X magnification. Several drops of 1 molar HCl (considered to be the standard for the geologic test) were applied, ensuring that soil and bone particles were covered/submerged. The observed reaction was categorized as being either non-effervescent or very slightly, slightly, strongly, or very strongly effervescent.

For all non-burned bone samples at both size fractions, the bone fragments in the soil were observed to be slightly effervescent. For the burned bone sample and hydroxyapatite standard, the particles were observed to be very slightly effervescent. In all cases, the bone particles could be easily identified within the soil based on the presence of small bubbles surrounding the bone particle. This is likely the result of calcium or phosphate in bone (as well as possibly organics) reacting with the HCl to yield a gas.

These results demonstrate that a simple, quick, cost-effective test can be used to determine whether soil may contain bone particles, even if they are too small to be identified morphologically. The possible forensic anthropological implications are significant since this method may be useful in identifying a possible burial (or previous burial) location. It also demonstrates that bone particles in soil can produce the same type of effervescence expected in carbonate soil and that additional tests (such as XRD) should be performed to determine the source of the reaction.

It is hoped that these results may form the basis for more sophisticated studies on using this type of test to detect the presence of skeletal material in soil. This study also highlights the insight that can be gained through interdisciplinary dialog and investigations.

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**Forensic Anthropology, Forensic Geology, Bone Detection**

## A170 Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part II—Intraskkeletal Histological Variability and Regional Differences

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**Learning Overview:** Attendees of this presentation will be introduced to novel histological data from a longitudinal live animal experiment documenting the effects of chronic opioid exposure on cortical bone remodeling. The ultimate goal is to describe how opioid agonists, particularly morphine sulphate and fentanyl, affect microscopic structures of cortical bone used in histological age-estimation methods in forensic anthropology. Related objectives include: (1) demonstrating intraskkeletal and regional variability in histologic parameters in the rabbit midshaft femur due to opioid exposure, and (2) presenting a novel toolkit (OsteoFlo®) for fluorescent osteon characterization.

**Impact Statement:** This presentation will impact the forensic science community by describing how opioid exposure dysregulates bone remodeling and the subsequent longitudinal impact on cortical bone microstructural parameters. Further understanding of such underlying biological processes will allow us to adapt histological age-estimation methods within the field of forensic anthropology for application to chronic opioid users.

Novel approaches to the study of changes in bone tissue are needed to explain the substantial variability observed in the pattern and pace of bone remodeling among diverse groups. Current histological age-estimation methods are limited by approaches that fail to account for underlying etiologies known to affect bone tissue (e.g., infection, stress, substance abuse, chronic alcohol use, medication regimes, hormones, among others). Previous studies have demonstrated that pathological conditions can affect bone remodeling and therefore age estimates.<sup>1-4</sup> Work by Karinen revealed that individuals who abuse methamphetamine were more likely to have their age underestimated (mean=-11.57 years) when evaluated using histological criteria.<sup>5</sup>

The purpose of this study was to develop a longitudinal model for evaluating the effects of prolonged opioid exposure on cortical bone remodeling in an animal model system. New Zealand White (NZW) rabbits were selected since they have a shortened remodeling period and relatively quick skeletal maturation. Male NZW rabbits were divided into three groups of seven animals each: morphine sulphate, fentanyl, and controls. In addition to eight weeks of experimental treatment, all animals underwent bi-weekly subcutaneous injection with a bone-labeling fluorochrome, calcein, to facilitate *ex vivo* dynamic histomorphometry following euthanasia. It was hypothesized that opioids would significantly alter microstructural features used in histological age-at-death estimation. Histomorphometric variables included mineralizing surface per bone surface, cross-sectional geometry, remodeling area, size and shape descriptors for vascular pores and osteons, intracortical remodeling activity, and activation frequency.

Mid-shaft bone blocks from rabbit femora were embedded in methyl methacrylate, and transverse thin sections were procured using a Well® diamond wire saw. Cortical bone microstructure was visualized using an Olympus® BX51 microscope, with cross-sectional images of each section acquired using Differential Interference Contrast (DIC) (200x), circularly polarized light (200x), and a Fluorescein 5-Isothiocyanate (FITC) filter cube for fluorescence (100x). A proprietary image processing toolkit (OsteoFlo®) was developed for FIJI software v.1.53k (6) that allowed for semi-automatic isolation, type classification, regional subdivision, and morphometric analysis of vascular pores and osteons. Statistical analyses were completed in R (v. 4.1.1), using Analysis of Variances (ANOVAs) to compare morphometric variables between drug groups and anatomical regions.

Whole bone analyses found that intracortical remodeling was significantly elevated in morphine rabbits and depressed in fentanyl rabbits, compared to controls. This pattern was observed in the cortical fraction of remodeled bone, active remodeling centers, and counts and population densities of osteon types. Regional subdivision additionally indicated that vascular pores and osteons were significantly concentrated posteriorly and reduced laterally. Osteons in lateral regions were also smaller. These results are consistent with repression of remodeling by high anterior and lateral strain during hopping. These results confirm the hypothesis that opioid exposure significantly dysregulates bone remodeling and its microarchitectural products.

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### Bone Remodeling, Opioids, Histomorphometry



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## A171 Post-Fire Modifications of Burned Human Remains

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**Learning Overview:** Attendees will understand some major factors that can alter burned human remains between the final moments of burning and the anthropological examination of the remains at the morgue.

**Impact Statement:** This presentation foregrounds several common post-fire changes that can occur to fragile burned human remains and bones during extinguishment, search and recovery, handling, transport, and the postmortem examination. Understanding these factors will allow the anthropologist to differentiate pre-fire trauma from post-fire defects.

Burned remains commonly seen by the anthropologist in the morgue have likely undergone post-fire changes due to activities from fire suppression, search and recovery, handling, transportation, and prior examination by the forensic pathologist and other personnel. By the time the anthropologist examines burned remains, it is essential to separate the damage due to: (1) pre-existing defects (pathology or trauma); (2) thermal changes; and (3) post-fire damage to burned bones. The San Luis Obispo Forensic Fire Death Investigation course has, over the past 12 years, utilized approximately 150 cadavers in realistic scenarios and provided the opportunity to identify these factors.

Fire suppression efforts focus on extinguishing the flames as quickly and safely as possible. One method to suppress the fire involves a pressurized column of water from the fire hose, which if the body is directly impacted, can cause the fragile charred and calcined bones to fragment and disperse within the fire scene, particularly for the head. Activities from heavy boot traffic and dragging fire hoses through the scene have the potential to cause damage if the body was in its path.

Search and recovery for fire victims often are premised on the belief that: (1) there is an intact body, and (2) this body is buried under the fire debris. One search technique is the “shuffle” method whereby the searchers shuffle through the debris in hopes of bumping into the body. If searchers are not trained to recognize charred and calcined human bone, it is likely that they could walk through the remains or focus on larger charred remnants and ignore or damage the surrounding bone fragments. Cadaver dogs alert to human remains by sitting or lying on the area of the greatest concentration of scent, often the pelvis. This can result in crushing of the spine or other adjacent skeletal elements.

Recovery of burned human remains from the fire scene involves safely gaining access, which may require extrication from vehicles with power tools or from heavily damaged structures with collapsed fire debris on and around the body. Excavation and removal of the fragile remains from their *in situ* context can produce additional fragmentation, especially from improper handling or untrained personnel (transport services). Recovery becomes challenging if charred tissues are fused to metal frameworks (car seat, bedsprings) or have adhering materials (melted glass, metal). Recovery can result in fragmentation if the body bag is placed next to the victim before sifting for bone fragments in the surrounding fire debris and from damage from boots of personnel moving the remains into the body bag. Body bags are flexible, which if lifted without a backboard, can produce pressure points against the fragile burned remains. Heavy-duty body bags can be flat and rigid, thus pressing against fragile bones of the face and teeth.

Transportation to the morgue involves strapping the body onto a gurney with 2- or 3-point belt restraints cinched against the fragile burned bones of the chest, flexed arms, and legs. Movements from loading, driving conditions, and unloading the gurney at the morgue can produce further fragmentation. At the morgue, the intake process can involve the transfer of the body to a new gurney/table, handling for radiographic images, removal of clothing/personal effects, and inherent damage from the invasive autopsy procedures that occur before the forensic anthropologist examines the remains.

Examples of damage from these factors will be presented to demonstrate the post-fire alterations that can occur to burned human remains from the fire scene to the morgue. Understanding these post-fire modifications will help isolate the areas of damage that may be most closely linked to the cause and manner of death.

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### Burned Bone, Fatal Fire, Burned Human Remains

## A172 The Effects of Experimental Whole-Body Burning on the Cortical Bone Microstructure of Ribs, Femora, and Metatarsals

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**Learning Overview:** This presentation will explain the effects of thermal alteration to the cortical microstructure and age-at-death estimates of ribs, femora, and metatarsals during whole-body burning experiments in simulated fire death scenarios.

**Impact Statement:** This presentation will impact the forensic science community by providing a better understanding of what to expect from histological analysis of thermally altered human remains at various temperatures based on realistic burning experimentation.

This project used willed body donations (N=6) with known demographics from the Forensic Anthropology Center at Texas State (FACTS), all of whom had consented to advanced/traumatic research, in various realistic fire death scenarios to understand the effects of thermal alteration to whole-body donations at the microstructural level. Midshaft samples of the sixth rib, femur, and fifth metatarsal were taken from one side of each donor before burning. For the duration of the thermal alteration, thermocouples were placed both in the body near the regions of interest (thorax, thigh, and foot) and throughout each fire scene to record temperatures. Depending on the scenario, the fires were allowed to burn between 13-53 minutes and the average temperature for each region of interest ranged from 666.66-864.87 °F. Post-burning, the antimer of each bone sample was taken from the other side of the body, if available. This resulted in 18 pre-burn and 15 post-burn samples in total.

Histological slides were made from each sample using standard histological protocols and evaluated on a Leica® DM6M microscope for general histological data collection and age-at-death analyses.<sup>1-4</sup> Standard histological taphonomic alteration indices were applied to both pre- and post-burn samples, including the General Histological Index (GHI), the Cracking Index (CI), and the Birefringence Index (BFI) to quantify visible changes.<sup>5</sup> A minimum of 40 osteons per sample were evaluated to compare changes in osteon area and Haversian canal area between pre- and post-burn samples.

Two-tailed *t*-tests were used to evaluate significant differences in index score, osteon area, and Haversian canal area within elements between pre- and post-burn samples. No significant differences in osteon area or Haversian canal area between pre- and post-burn samples were found. In the femur, significant differences in BFI were seen between pre- and post-burn samples ( $p<0.05$ ). In the metatarsal, significant differences in CI were seen between pre- and post-burn samples ( $p<0.05$ ). No significant differences in taphonomic indices were seen in the ribs. When pooling all elements, significant differences in BFI and GHI were seen between pre-burn ( $p<0.01$ ) and post-burn samples ( $p<0.05$ ).

The age estimate ranges from the femur across pre- and post-burn samples encompassed known age-at-death, provided enough cortical bone was preserved to include anterior width as an age estimate variable.<sup>2</sup> The age estimates for the rib were less accurate than the femur, but the point age estimates were not significantly different between pre- and post-burn samples.<sup>3,4</sup>

Overall, the results of this study show that thermally altered remains can be used for histologic analysis, including age-at-death estimation, within certain burning parameters. These parameters and practical guidelines for evaluating whether burned samples can be successfully used for histological analysis will be discussed.

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### Histotaphonomy, Burned Bone, Bone Histology

## A173 Estimating Exposure Time From a Set of Burned Human Remains

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**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the variables that can contribute to the timing and development of heat-related changes on a human body.

**Impact Statement:** This presentation will impact the forensic community by introducing a new tool that can be used in analyzing fatal fire cases and aid investigators in building a legal case.

Questions regarding timing of death events are often ones that investigators look to have answered, as they are not readily known. Within anthropology, a series of methods have been developed to provide an estimate of time surrounding death events. A majority of these models are derived out of taphonomic studies that focused on developing a Postmortem Interval (PMI).<sup>1-5</sup> Experimental observations have found that a human body undergoes a series of physical alterations at death, making it possible to estimate time since death.<sup>1-5</sup> This study aims to employ a similar approach and create a model for estimating exposure time to a fire, which can provide additional insight and information to forensic investigators building a legal case.

This study involves observational experiments of the burning of 80 donated human cadavers. Data were collected as part of the Fatal Fire Death Investigation Course by the San Luis Obispo Fire Investigation Strike Team, Inc. Cadavers were placed in vehicles, structures, confined spaces, and outdoor fire contexts. All physical alterations to both soft and skeletal tissues were documented with digital photography. Temperature data was collected through use of thermal couples and thermal imaging devices placed on multiple locations and depths directly on the human remains. The remains were assessed by applying a Total Body Score (TBS) based on the affected body region (i.e., skull, upper and lower limbs, thorax, and hands/feet). Once the visual analysis was completed, all scores for each region were added together to form a TBS and used in a predictive model to estimate exposure time to a fire.

This study found the amount of thermal damage to remains was correlated with maximum temperatures and exposure times in each fire environment. TBS and maximum temperature were found to be the most significant variables in predicting time, with each having p-values less than 0.001, which is statistically significant (p-value <0.05). Given these results, a linear regression model was created for estimating exposure time. The model correctly predicted time in about 70% cases analyzed. The model misclassified on the testing dataset in approximately 30% of the cases analyzed. While it did not exactly pinpoint the exact time, it was within range of 2-3 minutes in some cases, and at most off by 8-9 minutes in others, which is still a fairly acceptable rate of error. On average, the model was found to be off by 5 to 6 minutes, which is within the range of standard error reported. Despite outliers and a limited comparable sample, the model correctly predicted time in approximately 70% of cases analyzed.

The results of this study demonstrate the possibility to estimate exposure time from burned human remains. The findings also lend support to the notion that it is a combination of both time and temperature that influence bodily conditions. Overall, this model provides a solid foundation for future researchers to build upon, as there is currently no model like this one described for fatal fire deaths.

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### Burned Human Remains, Taphonomy, Forensic Anthropology

## A174 3D Analysis of the Microstructure of Heated Bone to Aid in Species Differentiation

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**Learning Overview:** After attending this presentation, attendees will better understand the microstructure of bone, how it differs between species, and understand the changes that occur upon heating.

**Impact Statement:** This research will impact the forensic science community by discussing a non-destructive, inexpensive technique that can be implemented to analyze the internal microstructure of bone three dimensionally to aid in the species identification of bone

Species identification of bone is the foremost question posed to forensic anthropologists upon the recovery of remains. This can be an extremely challenging task if anthropologists are provided with compromised remains. Highly fragmented burnt bone, for example, is notoriously difficult to identify as it is confounded by factors including warping, the breakdown of the organic component, fragmentation, and commingling with debris and other remains. Therefore, there is a need for a new method that can reliably determine the species of compromised bone. Cortical bone is comprised of rod-shaped cells that are tightly packed into concentric circular structures called osteons. Haversian (longitudinal) and Volkmann (transverse) canals facilitates blood vessels and nerve fibers to pass through the osteon, providing the bone cells with nutrients and enabling waste removal.<sup>1</sup> Cattaneo et al. and Martiniaková et al. have demonstrated the success of using Haversian canal measurements via histological examination as a species differentiating parameter; however, this method relies on 2D measurements and therefore does not account for changes in 3D space.<sup>2-4</sup> Further, the technique is destructive, with thin sections of specimens required for analysis. The project detailed in this abstract has utilized micro-Computed Tomography ( $\mu$ -CT) to analyze the 3D microarchitecture of the canal network of bone from bone specimens of different species of origin. This has provided an understanding on how these networks differ between species and how these changes are impacted by thermal alteration.

Bovine, canine, ovine, and porcine femoral cortical bone were cut into 5 x 5 x 5mm sections and scanned using a Nikon<sup>®</sup> HMX ST 225  $\mu$ -CT operating at 65kV and 70 $\mu$ A with a resolution of  $\sim$ 14 $\mu$ m voxel size. Three repeats of each sample were tested for each temperature ( $n=27$ ). The Haversian and Volkmann canals were separated from the cortical bone structure, and their volume and surface area were measured. The samples were then heated at 400°C, 600°C, and 800°C before being scanned again to compare any heat-induced changes. The results have shown significant differences ( $p < 0.05$ ) in the morphometrics of the Haversian and Volkmann canal networks between species. Overall, there is a reduction in the diameter and the frequency of the canals after heat treatment that is correlated with an increase in temperature. Crucially, after heat treatment, it is still possible to differentiate between species. These findings suggest that  $\mu$ -CT can potentially be implemented as a non-destructive tool to determine the species of origin of compromised bone.

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### Species Differentiation, Heated Bone, Bone Microarchitecture

## A175 Do Bone Elasticity and Postmortem Interval Affect Forensic Fractographic Analyses of Bone?

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**Learning Overview:** After attending this presentation, attendees will understand the effects of bone elasticity on fractographic features of bone fracture surfaces.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of forensic fractography into the recent postmortem interval.

The timing of tissue injuries informs cause and manner of death in forensic cases. Distinguishing perimortem and postmortem injuries is straightforward when soft tissue is present but may be challenging with skeletonized remains. Taphonomic events may obscure or mimic perimortem trauma, particularly when bone is still elastic. The prolonged perimortem interval in bone limits macroscopic fracture pattern analyses' capacity to discern perimortem injuries from postmortem damage.<sup>1</sup> Bone fractography characteristics of fracture surface features reliably established crack propagation in perimortem injuries, especially when used in conjunction with overall fracture patterns. This study used the fractographic approach described by Christensen et al. to evaluate experimentally induced perimortem and postmortem fractures.<sup>2</sup> The hypothesis was that the expression of fractographic features may differ as bone elasticity decreases, elucidating perimortem and postmortem events.

Fourteen unembalmed, defleshed human femoral shafts from males and females aged 33 to 81 years were included in this study; the periosteum was left intact. The bones were stored at -22°C and thawed at ambient temperature (20°C) for two days. Five femora were used as the perimortem sample, and nine femora were placed in a ThermoFisher™ Heratherm™ gravity convection oven at 35°C to simulate six postmortem intervals ranging from 1,500–16,000 Accumulated Degree Hours (ADH). A data logger was used to monitor and record temperature and humidity. Bones were weighed before and after removal from the oven to estimate water loss, and a 1cm sample was cut from each shaft for histochemical analysis of collagen. Next, the shafts were fractured with a Columbus McKinnon (CM) Drop Test Frame using three-point bending and drop height of 100–275mm. Fracture tests were recorded with a high-speed camera and Digital Image Correlation (DIC) was used to calculate stress and strain across the bony geometry, as well as displacement and location of failure. Fracture surfaces were scored according to descriptions provided by Christensen et al.<sup>2</sup>

A Multivariate Analysis of Variance (MANOVA) and polyserial correlations were used to measure associations between the fractographic variables, ADH, and collagen and water content of the bone. The MANOVA showed that ADH ( $F=0.99$ ,  $p=0.50$ ) and water loss ( $F=1.73$ ,  $p=0.23$ ) did not affect fractographic scores significantly. The polyserial correlation showed a strong negative correlation between cantilever curl and ADH ( $r=-0.86$ ,  $p=0.067$ ), and a moderate positive correlation ( $r=0.55$ ,  $p=0.45$ ) between bone mirror and ADH. The correlations were not significant, likely due to the small sample size and uniformity of some variable scores. The effect size is 0.96 (80% power,  $\alpha=0.05$ ), meaning that only a large effect would be observed with this sample size.

The hypothesis was rejected, demonstrating the potential utility of fractography into the recent postmortem interval (up to 16,000 ADH). The polyserial correlation results and the effect size indicate the need for further investigation with a larger sample and longer postmortem intervals.

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### Bone Fractures, Forensic Fractography, Accumulated Degree Hours

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## **A176 The Value of Antemortem Skeletal Data in Interpretation of Acute Trauma: Context, Timing, Mechanism, and Likelihood Risk Assessment**

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**Learning Overview:** This research highlights the value of antemortem skeletal observations and data in understanding and interpreting acute (perimortem) trauma. Attendees will learn how antemortem conditions, including evidence for pathology and fracture, can inform our interpretations of acute trauma, its timing, mechanism, and context.

**Impact Statement:** Antemortem data can provide forensic anthropologists and other forensic practitioners with additional information beyond identification. Knowledge of these conditions can provide contextual understanding of perimortem traumatic events, their timing, and mechanism; they can also potentially be used to generate risk assessments for quantitative interpretation of the likelihood of such events.

Analysis of antemortem skeletal conditions has traditionally been of fairly limited use in forensic anthropology, primarily centered on identification of a decedent. This presentation highlights the value of antemortem conditions beyond the realm of forensic identification. Antemortem conditions, particularly those related to traumatic events like fracture and/or disease, typically exhibit evidence of a biological (osteological) response, and consequently hold much potential for understanding the context, timing, and mechanism of perimortem events. Pre-existing skeletal trauma, in some cases, reflects repeated (serial) traumatic episodes and, it is hypothesized, is associated with a higher risk of negative outcomes.

A review of 261 forensic cases from the Radford University Forensic Science Institute (RUFISI) case files identified 12.63% ( $n=33$ ) that were associated with antemortem conditions of probative value. These primarily fell into the following categories:

Suspicious child deaths for which a repetitive (serial) pattern of fracture was documented and its mechanism (non-accidental injury) supported. This patterned evidence was often only evident through microscopic documentation of a healing (i.e., osteoblastic, osteoclastic) response. It was the key to determination of the timing of events and, ultimately, accurate adjudication of these cases. This was also evident for adult and elderly domestic abuse cases, in which repetitive fracture events in the thorax, upper limbs, and face and even hyoid were prominent.

Documentation of antemortem comorbidities and pathological conditions (e.g., alcoholism, diabetes, osteoporosis) informed the interpretation of perimortem events with regard to susceptibility to accidental fracture.

Understanding the probability of these events based on these priors can increase their probative value and assist with perimortem interpretations of the context, mechanisms, and timing of acute trauma. The use of maximum likelihood ratios in estimating acute trauma mechanisms and quantifying trauma risk is explored. It is recommended that antemortem data in the form of observations of skeletal trauma and evidence of disease be collected and applied toward understanding, interpreting, and possibly predicting acute trauma.

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### **Antemortem, Trauma, Acute Trauma**

## A177 tRauma: A New Application for Aiding in Blunt Rib Trauma Interpretations

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**Learning Overview:** After attending this presentation, attendees will be familiar with a new Graphical User Interface (GUI), tRauma, which is available for aiding in analysis and interpretation of rib trauma through heat map analysis and robust statistical analyses.

**Impact Statement:** This presentation will impact the forensic science community by providing statistical substantiation of a practitioner's interpretations of rib trauma, which can help allow trauma analysis to adhere to the *Daubert* criteria and meet the suggestions of the National Academy of Sciences Report from 2009, which pushes for "good science."<sup>1</sup>

Trauma analysis is the most common reason that forensic anthropologists provide expert witness testimony, which is continually increasing in demand.<sup>2</sup> Within a medical examiner's setting, the most common trauma observed is blunt force trauma, specifically to the ribs and cranium.<sup>3-5</sup> However, trauma analysis methods and interpretations of blunt force trauma to the ribs remain heuristic and lack sufficient predictive methodologies.<sup>6</sup>

It is difficult to interpret rib fractures under the same paradigm used to interpret trauma in long or flat bones. First, ribs are structurally unique from all other flat bones and long bones, and therefore behave differently mechanically under strain. Second, ribs rarely are isolated at time of impaction, and their articulation with the rest of the rib cage causes interactions that can potentially mitigate applied strain. As a result, many practitioners forego interpretation of rib trauma, which may cause loss of information that could contribute to overall interpretation of the death event.

An open access GUI, called tRauma, was created to combat the difficulties associated with blunt force trauma to the ribs. The GUI utilizes a large-scale database of documented rib fractures from geographically diverse medical examiner offices. Each individual included in the database has documented Cause and Manner Of Death (COD, MOD), as well as demographic information, such as height, weight, sex, age, ancestry, and associated health variables (i.e., obesity, pathological conditions, etc.). Every fracture was documented for each individual and included associated details, such as rib number and location, completeness, and fracture type.

tRauma provides three main functionalities: (1) database manipulation that provides heat maps of rib trauma distributions and patterns, (2) fracture location input that provides database cases with similar trauma patterns, and (3) robust statistical analyses to classify a new case based on database information. Database manipulation functions include filtering by associated demographic, fracture, MOD, and COD-related information, or selecting which columns the user considers pertinent for a search. Fracture location input allows for documenting one or all fracture locations of a single case. Finally, statistical analyses provide substantiated statistical support for the user's own rib trauma interpretations.

tRauma can be utilized to help practitioners provide rib trauma interpretations with statistical substantiation, regardless of experience level. This open access resource has the potential to be used as an interpretive tool for rib trauma interpretation based on real-world scenarios that can be directly applied in most instances of forensic anthropological casework.

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### Rib Trauma, Trauma Analysis, Graphical User Interface

## A178 The Frequency of Wedge Fractures in Experimental Bending of the Tibia

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**Learning Overview:** After attending this presentation, attendees will understand the importance of experimental research in skeletal trauma analysis and interpretation through an examination of fracture pattern frequencies in a single loading condition.

**Impact Statement:** This presentation will impact the forensic science community by presenting experimental skeletal trauma fracture characteristic data to evaluate current assumptions regarding fracture patterns and associated loading mechanisms.

The majority of expert testimony provided by forensic anthropologists is analysis and interpretation of skeletal trauma. However, there are still major gaps between current knowledge, skeletal trauma research, and judicial standards of scientific validity. General assumptions regarding fracture patterns and their association with specific loading mechanisms prevail within the field of forensic anthropology. As such, concepts such as wedge fractures are the result of bending and oblique fractures are the result of angled compressive loading are perpetuated without controlled experimental bone trauma data to support their reliability. In order to provide quantifiable methods to support expert testimonies regarding trauma analysis and reconstruction of traumatic events, experimental research conducted on large sample sizes of human specimens employing interdisciplinary methodologies must be utilized. The objective of this study was to test the assumption that a dynamic bending loading mechanism results in wedge fractures.

Fractures of the tibial shaft are the most common of all diaphyseal fractures, specifically, injuries are more common in the middle and distal portions.<sup>1-3</sup> The tibia is frequently fractured in pedestrian versus motor vehicle crashes, motorcycle crashes, sports injuries, and high-speed activities.<sup>2,4</sup> Therefore, all controlled experimental blunt force trauma testing for this study was conducted on the diaphyseal region of the human tibia. The sample for this study consisted of 50 human tibiae (25 females, 66.6 years  $\pm$  22.1; 25 males, 58.6 years  $\pm$  21.2) collected from Postmortem Human Subjects (PMHS) and frozen in saline-soaked gauze until testing to prevent dehydration. Prior to testing, all tibiae were potted at the 20% and 80% sites using an anatomical coordinate system to ensure the same orientation across all tests. All tibiae were impacted dynamically in the lateral-medial direction at 6m/s at the 40% and 60% sites, simultaneously, in a four-point bending scenario. Fracture characteristics of each tibiae were recorded and fracture classifications were determined utilizing the Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association (AO/OTA) Fracture and Dislocation Classification system.<sup>5</sup>

Of the 50 tibiae impacted, the following number of fractures per tibiae were observed: one ( $n=23$ ), two ( $n=16$ ), three ( $n=6$ ), four ( $n=4$ ), and five ( $n=1$ ). To control for the possible influence of additional fractures, further evaluation of fracture groups and types was limited to tibiae that resulted in only one fracture. Fracture groups and types observed were simple oblique ( $n=3$ ), intact wedge ( $n=6$ ), fragmentary wedge ( $n=5$ ), intact segmental ( $n=2$ ), and fragmentary segmental ( $n=7$ ). While wedge fractures (intact and fragmentary) were the most common fracture type observed (48%), more than half of the sample (52%) resulted in fractures other than wedge; segmental fractures (39%) and simple fractures (13%). Fifty human tibiae were impacted in precisely the same loading direction at the same rate, yet, there was variation in both the number of fractures per tibia and fracture classifications, demonstrating that loading mechanism should not be assumed based on fracture group and type.

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### Skeletal Trauma, Fracture Classification, Trauma Analysis



## A179 Correlation or Causation? Concealing the Victims and Survivors of Domestic Violence

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of domestic violence as it intersects and aligns with the Forensic Anthropologist's (FA's) role in a medicolegal death investigation.

**Impact Statement:** This presentation will impact the forensic science community by adding to the existing literature that supports the wider scope of the FA.

Forensic anthropology literature inconsistently defines the scope of FAs in their role in the identification of the Domestic Violence (DV) etiology with respect to trauma analysis. Historically, FAs remained "behind the curtain," solely evaluating the skeletal trauma, while neglecting the obvious, often pervasive, social problems and mechanisms that may be linked to such traumata. Opponents argued that correlation implies causation and that the FA should avoid opining specific antemortem changes consistent with DV.

However, more recently, researchers infer DV etiologies by evaluating skeletal trauma and circumstantial evidence from a holistic perspective.<sup>1,2</sup> Proponents of the holistic perspective imply that correlation does not necessarily equate to causation, but that the correlation should be considered. Indeed, clinicians and medical examiners utilize circumstantial data to reveal clues relevant to diagnoses or cause and manner of death determinations. This study provides data to support and validate the FA's responsibility to identify DV etiologies in order to meet the expectations of a modern society and modernized science, especially mid- and post-COVID-19 pandemic, and with the recent dissolution of agencies such as the Florida Coalition Against Domestic Violence (FCADV).

In this study, 117 cases of blunt force trauma were evaluated and parsed between male and female cohorts. Generally, these data revealed that *only antemortem trauma* (e.g., to the exclusion of acute perimortem trauma) was the most common trauma category between male and female cohorts. However, when parsed by sex, the *only antemortem trauma* and *only perimortem trauma* categories were equally as common in females ( $p=0.004901$ ). DV was identified through the FA and medicolegal death investigations and was more common in females ( $p=0.001044$ ), consistent with clinical, psychological, forensic, and sociological literature.<sup>3,4</sup> Of the 23 females represented in this study, 15% had medical documentation to inform the antemortem trauma, whereas 63% of males ( $n=94$ ) had similar documentation. These statistical analyses were performed using R-Studio Statistical Analysis software.

These findings support the need for an investigation of antemortem injuries, especially when the cause of death is unknown. This study also highlighted that the FA tends to be called in for a focused perimortem trauma analysis by the legal authority rather than to conduct holistic trauma analysis that would include healed antemortem injuries on skeletal remains not included in the focused analysis. More specifically, the FA may be asked to evaluate a portion of a decedent's body, leaving the antemortem trauma either undetected or unexplained. This study also highlighted the potential for DV to remain hidden in the decedent's lifeways, carefully captured in their skeletal record. These findings suggested that full-body radiographs should be mandatory in death investigations and that FAs should more consistently acknowledge DV as an etiology. Otherwise, these latent, or simply unidentified, wounds can further silence and conceal a victim or survivor of domestic violence.

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### Domestic Violence, Blunt Force Trauma, Death Investigation

## A180 Indirect Gunshot Trauma to the Skeleton: A Meta-Analysis and Experimental View

Samantha Coberly, PhD\*, SNA International, Honolulu, HI

**Learning Overview:** The purpose of this research is to better understand the circumstances and frequency of indirect trauma in the skeleton. This presentation will allow attendees to recognize where indirect trauma from Gunshot Wounds (GSW) occurs and under what circumstances so that if elements of direct trauma are missing, it may be possible to still identify GSWs.

**Impact Statement:** This presentation will impact the forensic science community by allowing for better recognition of indirect trauma.

Indirect trauma occurs when the element was not hit directly by a projectile, but sustained damage due to the forces involved from the projectile.<sup>1-4</sup> As the projectile moves through the body, forces pulse through the tissue as a transfer of energy, taking several forms such as shock waves and temporary cavities. This cavity is thought to be responsible for indirect trauma.<sup>2-6</sup> The temporary cavity, if strong enough, can cause tissue damage far removed from the area of impact.<sup>7,8</sup> The purpose of this research is to better understand the circumstances and frequency of indirect trauma in the skeleton.

C.A. Pound Human Identification Laboratory (CAPHIL) cases between 1974 and 2020 were examined for indirect GSW trauma through case records/reports/photos and focused on cases with clear entrances and exits. Out of 263 total GSW cases, 29 (24.7%) had indirect trauma. Sixty-five total elements exhibited indirect trauma, with an average of 2.07 per case. Vertebrae, ribs, sternum, and scapula were represented in the sample; vertebrae (32; 49%) and ribs (31; 47.6%) were the majority. With the exception of one case, the indirect trauma was limited to articulating elements only. Twelve cases had known weapons; 58% (7) were handguns.

In addition, eight pigs were shot in the lateral thorax with a handgun; four with a .22cal bullet (one pig was shot twice making five shots in total) and four with a 9mm bullet (four shots in total). The pigs were defleshed, macerated, and analyzed for trauma. All the pigs shot with the .22cal had no indirect trauma. Three of the 9mm pigs had indirect trauma. The indirect trauma was near the element directly hit. The majority were ribs.

Elements with indirect trauma in the CAPHIL sample exhibited multiple trauma patterns: avulsion, incomplete fractures, damage to facets, processes, lamina, pedicles of vertebrae, and bisection of ribs. Comminution was not present in vertebrae but was in ribs. The indirect trauma was less than the elements that were hit directly. The same pattern can be seen in the pig experiment with the 9mm handgun; multiple ribs having clean, complete fractures to the sternal end. Several ribs had similar fractures to the head and neck as well.

Within the literature, indirect trauma is not often discussed in detail, but the possibility is mentioned.<sup>8-14</sup> Indirect trauma tends to be simple; sharp with clean fractures and rarely comminuted. Oblique, spiral, and linear fractures can occur. Overall, indirect trauma is non-specific.<sup>2-4, 15-18</sup> This was consistent with the findings of this study. Typically, vertebrae exhibit minor damage to processes and lamina and ribs have damage to neighboring ribs.<sup>17</sup> Although not seen in this study, long bones can exhibit wedge-shaped fractures.<sup>3,4</sup> One study found that projectiles that caused trauma passed on average 9.68mm from the bone during their trajectory.<sup>2</sup> Unfortunately, the limitations of the study do not allow for interpretation of distance, but it is logical that the closer to the bone, the more damage is present.

In the literature, indirect trauma has typically been associated with higher-powered weapons, especially rifles.<sup>9,11,12</sup> The current study found indirect trauma with shotguns, handguns, and rifles. There were two cases in the CAPHIL sample where there was indirect trauma from a .22cal, although pigs shot with the .22cal did not exhibit this. Trauma was also not found far from the wound track or from where there was a direct hit from the projectile, except in one case. Fractures to adjacent bone may result from smaller waves of forces dispersing through the bone rather than directly from the temporary cavity. Further study is needed before conclusions can be drawn. With current research and future study, anthropologists can start to predict when and where indirect trauma will occur. Overall, indirect trauma is generally less than direct trauma, can occur in a variety of scenarios involving multiple types of elements, and can occur with different types of firearms.

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### **Gunshot Wound, Indirect Trauma, Fractures**

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## **B1 Development and Validation of the InnoXtract™ DNA Extraction Method for Bone and Teeth Samples**

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**Learning Overview:** After attending this presentation, attendees will understand the development and utility of a novel extraction and bead purification kit designed specifically for the extraction of degraded DNA from compromised bone samples, demonstrating the potential use of this new technology.

**Impact Statement:** This presentation will impact the forensic science community by providing a new tool for degraded DNA extraction from bone and tooth samples. The method can be automated for high throughput and help to provide further benefit in solving missing person and forensic cases.

InnoXtract is a nanoparticle magnetic bead-based DNA extraction method with optimized digestion and binding buffers for high recovery and reproducible DNA yield from highly degraded forensic samples such as hair shafts and bone. We report here an optimized method for demineralization and digestion of 40 mg of bone and tooth samples for purification of DNA utilizing the high yield InnoXtract DNA purification method. The InnoXtract method was developed to address challenges commonly seen in bone samples, including low-template, highly degraded, and/or inhibited DNA. Forensic crime laboratories receive bone and teeth as evidentiary samples and process them for DNA evidence as a means of identification of individuals. Oftentimes, nuclear DNA is too scarce and degraded to enable sufficient profile recovery from bone using standard methods. Even with current Short Tandem Repeat (STR) kits available on the market, the ability to obtain meaningful profiles may be limited due to the low amount of recovered nuclear DNA. In most cases, laboratories will potentially try to extract mitochondrial DNA (mtDNA) from the bone as there are hundreds or thousands of copies per cell compared to the nuclear DNA. However, the process of recovering, processing, and analyzing the mtDNA can be labor intensive and time consuming on resources in the forensic laboratory. This presentation presents an extraction kit with specially designed buffers and magnetic beads capable of recovering fragmented and low-level DNA from samples such as bone. This will save time and effort on the part of the lab as it fits into the standard workflows of forensic laboratories and has the capabilities of producing greater DNA quantities and more successful STR profiles for difficult sample types. Optimization studies included improved conditions to pretreat bone and teeth to allow for complete digestion. Validation studies were performed to evaluate sensitivity, stability, repeatability and reproducibility, non-probative case type samples, contamination and method comparisons adhering to the Scientific Working Group on DNA Analysis Methods (SWGDM) guidelines. The InnoXtract extraction method yielded ample DNA (over 800 pg) from severely insulted bone and teeth samples to produce interpretable autosomal STR and InnoTyper 21 profiles. The method is also amenable to automation and high-throughput sample processing.

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### **DNA Extraction, Bone, InnoXtract™**

## **B2 The Effects of Temperature and Precipitation on the Amount of Recoverable Human DNA From Soil During Decomposition**

*Wesley Wagner\*, Duquesne University, Pittsburgh, PA; Pamela Marshall, PhD, Duquesne University, Pittsburgh, PA*

**Learning Overview:** From this presentation, attendees can hope to learn about how environmental factors (temperature and precipitation) can affect the quality and amount of DNA that can be extracted from soil during human decomposition.

**Impact Statement:** This project hopes to impact the forensic science community by attempting to find a relationship between the amount of recoverable DNA and certain environmental factors: temperature and amount of precipitation.

When it comes to human decomposition, various studies have focused on any number of variables, including, but not limited to, temperature, soil pH, soil type, insect activity, medical conditions of deceased, soil moisture, weather patterns, scavenging activity, and many others. The two factors that are largely seen as contributing the most to the decomposition processes are temperature and soil moisture; however, these studies largely focused on the rates of decomposition of the sample.<sup>1,2</sup> This project aims to expand the focus from the decomposing sample to the soil surrounding it. More specifically, this project prioritizes the presence of DNA that has leached into the soil by the decomposing sample, and what effect the two aforementioned variables, temperature and soil moisture, have on the amount and purity of recoverable DNA. To accomplish this, 18 equal sized human tissue samples will be buried into cloth grow pots and will be set to decompose outdoors. While decomposition is occurring, both temperature and soil moisture (in the form of precipitation) will be recorded at the experiment site. At one, three, five, seven, nine, and twelve months, three of the samples will be removed, and the soil immediately around the sample will be collected. The amount and purity of DNA extracted from the soil samples will be quantified using a Nanodrop. It is hypothesized that the amount of DNA recoverable from soil during human decomposition will rise along with increases in temperature and will also increase with higher amount of precipitation and humidity.

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### **Decomposition, Soil, DNA Extraction**

## **B3 Extraction Efficiency Testing of Degraded Bone Samples: Comparing Two Extraction Methods for Use in Downstream Massively Parallel Sequencing (MPS) Applications**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the need for an extraction protocol that maximizes recovery of small fragments of DNA for compromised forensic bone samples for potential use with massively parallel sequencing applications.

**Impact Statement:** This presentation will impact the forensic science community by providing information on the differences in DNA recovery and performance of two DNA extraction methods.

In recent years, investigative genetic genealogy (IGG), which involves the use of genealogical methods combined with DNA analysis to generate potential familial matches, has become an important tool which applies massively parallel sequencing (MPS) in solving cold and active cases.<sup>1</sup> Traditionally, forensic DNA amplification methods have relied on the need to amplify large (150-500 base-pair) targets, specifically, short tandem repeats (STRs) for forensic profiles.<sup>2</sup> With MPS, smaller targets, such as single nucleotide polymorphisms (SNPs), have become useful for identification as well as ancestry and phenotype prediction.<sup>3,4</sup>

Traditional commercial extraction protocols for forensic DNA have not focused on maximum recovery of small fragments, whereas ancient DNA (aDNA) research has shown evidence for increased recovery of even highly degraded samples in excess of one million years.<sup>5,6</sup> When DNA yields are expected to be low from skeletonized remains, it is crucial that the implemented DNA extraction technique will maximize the quality and quantity of the DNA extracted. Given the new application of MPS in forensic DNA workflows, a comparison of forensic and ancient DNA extraction techniques was merited.

This study analyzed the quantity and quality of DNA recovered from two extraction methods involving degraded bone samples. A modified protocol as described in Rohland *et al.* was used for the aDNA extraction technique, while the PrepFiler® BTA Forensic DNA Extraction Kit from Applied Biosystems was used for the forensic extraction method.<sup>7,8</sup> Seven humeri, obtained with permission from Western Carolina University's John A. Williams Human Skeletal Collection, were used in this study. A total of 100 mg of bone powder were collected from each bone sample, with 50 mg from each bone sample designated for each method. Samples were quantified using the Quantifiler™ Trio Quantification Kit from Applied Biosystems.<sup>9</sup> All statistical analyses were performed in R (version 4.0.4) and Microsoft Excel.

One-way ANOVA tests were performed to compare small target DNA concentration and large target DNA concentration yields from both extraction methods. The results from the small target ANOVA test showed that there was no significant difference in the yields from both methods ( $F_{1,12}$ ,  $p = 0.418$ ). In addition, the results from the large target ANOVA test were similar, in that there was no significant difference in the yields from both methods ( $F_{1,12}$ ,  $p = 0.465$ ). Both methods performed similarly in their abilities to remove PCR inhibitors and in degradation indices. Further testing with a larger sample size is needed for more conclusive results.

The goal of this research is to determine the optimal DNA extraction method for maximum recovery of small DNA fragments in skeletal samples of forensic age for downstream MPS applications.

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### **Massively Parallel Sequencing, DNA Extraction, Human Identification**

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## **B4 Evaluating the Effectiveness of Microbial DNA Extraction Kits for Intimate Samples**

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**Learning Overview:** After attending this presentation attendees will have a better understanding of the vaginal and penile microbiomes and how they can potentially relate to sexual assault cases.

**Impact Statement:** This presentation will impact the forensic science community by presenting foundational data for the potential use of microbial DNA as a complementary type of evidence in sexual assault cases when sperm is absent from the evidence.

When a sexual assault is reported, semen (containing sperm cells) collected via intimate swabs during a sexual assault kit examination can support a claim that a sexual act occurred and can potentially be tied to a specific individual using DNA typing.<sup>1</sup> However, collection and processing of sperm evidence may not be possible in cases such as when the sexual assault did not result in ejaculation, barrier contraception such as a condom is used, the perpetrator having a low sperm count, or has had a vasectomy.<sup>2</sup> In addition, potential victims may delay reporting an incident, or shower prior to the sexual assault exam, reducing the success of retrieving adequate amounts of biological evidence for DNA typing.<sup>3</sup>

Therefore, alternate types of evidence such as microbiota from male and female genital areas would be beneficial in the absence of sperm. In regards to sexual assaults and the human microbiome, the vaginal and penile microbiomes are the two most important body sites in heterosexual cases. However, little is known about the microbiome of these sites and their potential application in some sexual assault cases.

The aims of this research were to evaluate the effectiveness of five commonly used commercial DNA extraction kits used to purify DNA from a microbial biomass and to further establish the baseline vaginal and penile microbiomes. Five commercial microbial DNA extraction kits (Invitrogen® Purelink™ Microbiome DNA Purification Kit, PDQeX prepGEM Bacteria Kit, QIAGEN® QIAamp® DNA Mini Kit, QIAGEN® DNeasy® Power Soil Kit, and QIAGEN® QIAamp® DNeasy® UltraClean® Microbial Kit) were evaluated. The variation in bacterial composition across donors was also explored as well as identifying unique bacteria to donors.

Overall, the Invitrogen® Purelink™ Microbiome DNA Purification Kit was found to be the best performing DNA extraction kit as it recovered the highest amount of DNA across the representative microbial populations. This method was then used for a proof-of-concept study with male and female intimate samples (N = 10 of each) to demonstrate the microbiota normally present in the penile and vaginal microbiomes using next generation sequencing technology to target the V3 region of the 16S rRNA gene. A wide range of microbial genera and species were seen across all male donors. However, all the female samples had a very high relative abundance of the *Lactobacillus* genus (>90%). Additionally, unique genera and species were identified in most donors demonstrating the potential use of microbial evidence for identification purposes.

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### **Sexual Assault, Human Microbiome, 16S rRNA**

**B5 The Development of a Fully Automated System Employing Parallel Processing for Highly Efficient Extraction and Purification of DNA From a Range of Challenging Samples**

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**Learning Overview:** The work presented here will provide attendees an understanding of a newly developed DNA extraction and purification system that is capable of improving sample processing efficiencies in a range of potential settings.

**Impact Statement:** This work will impact the forensic community by introducing a new method for efficient and versatile extraction of DNA from samples.

A system that combines a highly robust extraction chemistry with medium/high-throughput automated liquid handling and a DNA extraction/purification workflow capable of efficiently processing a range of challenging samples is presented here. Key to this platform minimizing runtimes is its ability to execute liquid handling steps and extraction/purification steps in parallel -- 96 samples can be processed on this system in approximately 80 minutes. In addition to performance on challenging bone, tooth and adhesive containing samples, also demonstrated here is the ability of this combined system to effectively process certain frequently encountered case-type samples, particularly mock touch/trace samples.

In studies on challenging bone, tooth and adhesive-containing samples, this platform delivered results that were comparable to those seen with a well-established on-market automated DNA purification instrument that has significantly less throughput capacity (13 samples per run versus 96 samples per run, with both systems employing offline lysis). Good repeatability and reproducibility over two independent experiments on this platform was demonstrated with a challenging bone sample, as well as good correlation to performance seen on the existing lower throughput platform, with respect to DNA yields, and downstream STR profile quality and completeness. Additional experiments with tooth and adhesive-containing samples, as well, returned results very consistent with the above-described findings. Further evidenced in these studies was similar comparative performance capabilities with a range of mock touch/trace case-type samples. As a test of the sensitivity of this system on a common body fluid/substrate pair, a sample set consisting of a dilution series of dried blood spotted on cotton swabs was processed. Full STR profiles were consistently obtained with as little as 0.63  $\mu\text{L}$  of blood input, and either full or almost full profiles were seen down to 0.16  $\mu\text{L}$  blood input. Importantly, over the diverse sample set examined in this validation study, including swabs of blood stains on denim, there was no indication of inhibitory factor carry-through in any of the eluates.

In conclusion, on a set consisting of 96 case-type sample lysates, the combined system described here can deliver results highly comparable to those seen with a medium/low throughput platform in significantly less time and with output plasticware options, such as 96 well PCR plate, that are more efficient for typical downstream processing.

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**Automation, DNA, Extraction**



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## **B6 A Comparison of Five DNA Extraction Methods to Extract Genomic DNA From Human Body Fluid and Fly Artifact Samples**

*Alexis Garloff\*, Towson University, Towson, MD; Kelly Elkins, PhD, Towson University, Towson, MD; David Rivers, PhD, Loyola University Maryland, Baltimore, MD*

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**Learning Overview:** After attending this presentation, attendees will better understand the impact of DNA extraction methods on DNA recovery and STR DNA profiling from human body fluid samples and fly artifacts deposited by flies fed on human body fluids.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees about the results of a study comparing different methods of DNA extraction for human DNA typing success from body fluid samples and fly artifacts deposited by flies fed on human body fluids including blood and semen.

Human identification is integral to forensic investigation. Environmental conditions, surface porosity, and evidence type can influence DNA recovery from submitted evidence samples. Numerous methods have been shown to successfully extract high quality and sufficient quantity of DNA from human body fluids for STR DNA typing to aid in investigations. In this study we evaluated four methods to extract human DNA from human body fluid samples and *Calliphora vicina* fly artifact samples. The fly artifact samples include fly defecate and regurgitate after the flies fed on human blood or semen and were sampled over several days and weeks. This study analyzed the results of quantitative PCR DNA yield and degradation index, UV-Vis spectroscopy DNA quality estimate, and STR DNA profiles generated from four different DNA extraction techniques.

**Methods and Discussion:** This study obtained DNA from body fluid and fly artifact samples using four different DNA extraction methods: Qiagen EZ1<sup>®</sup> DNA Investigator<sup>®</sup> kit, Monarch Genomic DNA Purification Kit, Zymo Quick-DNA Miniprep Plus Kit, and the prepGEM Universal DNA Extraction Kit. Three 1.2 mm punches were isolated from filter paper and combined for the human body fluid blood and semen control samples and fly artifacts in triplicate. The DNA quantity and degradation index was measured using the Qiagen Investigator<sup>®</sup> Quantiplex<sup>®</sup> Pro RGQ Kit and the quality was estimated using the 260/280 nm absorbance ratio acquired using a Thermo Fisher NanoDrop spectrophotometer. The average and standard deviation of the DNA recovered from each sample type was tabulated. STR profiles were generated using Qiagen's<sup>®</sup> Investigator<sup>®</sup> 24plex QS kit, a kit designed to amplify 24 loci including the expanded Combined DNA Index System (CODIS) core loci and European Standard Set (ESS) loci for a total of 22 STR loci, amelogenin, SE33, and DYS391. STR amplicons were separated and analyzed on an Applied Biosystems 3500 capillary electrophoresis instrument. The analysis suggests that DNA extraction methods that perform best isolate the DNA from other cellular material and concentrate the DNA prior to elution. Substitution of different buffers can improve DNA yield in some cases. Careful selection of the DNA extraction method can improve the DNA yield and STR profile completeness in forensic case analyses where other methods have failed. Partial STR profiles were obtained from the *Calliphora vicina* fly artifacts. For future studies, whole genome amplification prior to STR typing may enable us to obtain more complete profiles.

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### **DNA Extraction, STR Profiling, Fly Artifacts**

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**B7 A Comparison of DNA Extraction Techniques for the Recovery of Bovine DNA From Maggot Crops**

*Cesar Cantu, BS\*, Sam Houston State University, Huntsville, TX; Sibyl Bucheli, PhD, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** This presentation will provide attendees with information regarding different DNA extraction methods that can be used to recover host DNA, in this case, bovine DNA, from the crops of maggots. Assessment of the DNA extraction methods was performed by measuring the bovine DNA, quantified by qPCR, and the quality of the extract by assessing inhibition. The relation between the recovery of DNA from maggots and the quality and quantity of extract is essential for using maggots as samples for forensic casework.

**Impact Statement:** The use of maggots as a potential sample for human identification has been shown to be possible to recover human mitochondrial DNA and short tandem repeats (STRs) from the crops of a maggot. This project aids in the implementation of maggots as a sample type that can be useful in unique cases in which a body is not present, or other samples are not available. However, to successfully use maggots in forensic laboratories, an optimal DNA extraction method must be determined. This study works toward that goal by evaluating different DNA extraction methods based on the quality and quantity of DNA recovered.

Forensic entomology aims to aid criminal investigations through the use of insects, and it is primarily associated with estimation of the postmortem interval (PMI). Another potential use of insects is the recovery of human DNA, for example, from the digestive tracts of larvae of Diptera (maggots) found on decomposing bodies. Studies have shown that human DNA can be recovered from the crops of maggots, making them useful in identification of individuals when tissue is not available or in establishing a link between a scene and a victim. While several factors regarding the recovery of human DNA from crops have been studied, DNA extraction methods have not been thoroughly assessed. Determining an optimal extraction method could aid crime laboratories in using human DNA extracted from maggot crops. In this study, four DNA extraction methods were chosen to extract bovine DNA from maggots: a silica-coated magnetic bead, silica column, enzymatic lysis, and a chelating agent method.

Bovine DNA was targeted as a substitute for human DNA to test the above listed DNA extractions kits. Fly traps with beef heart were placed at Southeast Texas Applied Forensic Science (STAFS) Facility and then moved to incubators once eggs and maggots were observed to rear maggots to the third larval stage (instar). Maggots were identified to the family level prior to DNA extraction. Four DNA extraction methods (Chelex<sup>®</sup>, PDQeX forensicGEM, EZ1<sup>®</sup> DNA Investigator, DNeasy<sup>®</sup> Powersoil<sup>®</sup> Pro Kit) were evaluated based on the quantity of bovine DNA and quality of extract. Extractions were performed on whole maggots and dissected crops. Quantification was performed using real-time PCR (qPCR) on a StepOne<sup>™</sup> Real-Time PCR System with SYBR<sup>®</sup> Green using bovine specific cytochrome b primers. The quality of extracts was determined by checking for inhibition using commercial qPCR chemistries with an internal PCR control (IPC). When using whole maggots, Powersoil<sup>®</sup> Pro (n=10, 0.668± 0.458 ng/μL) yielded the highest average DNA yield, while EZ1<sup>®</sup> DNA Investigator (n=10, 0.605± 0.403 ng/μL) yielded the highest average from isolated crops. Chelex (Crops: n=10, 0.029± 0.033 ng/μL) and forensicGEM (Crops: n=10, 0.021± 0.032 ng/μL) yielded low amounts of bovine DNA, and DNA extracts were inhibited, unlike EZ1<sup>®</sup> and Powersoil<sup>®</sup> Pro, which have purification steps. It is recommended to use EZ1<sup>®</sup> DNA Investigator coupled with automation on EZ1<sup>®</sup> Advanced XL to recover DNA from maggot crops.

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**Forensic Entomology, Maggots, DNA Extraction**

## B8 Magnetic Bead Capture of Sperm Cells by Sperm-Specific Antibodies

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**Learning Overview:** Upon attending this presentation, attendees will gain insight into the forensic applications of immunomagnetic cell isolation.

**Impact Statement:** This presentation will impact the forensic science community by presenting support for novel applications of sperm-specific antibodies in the use of testing sexual assault evidence.

Intimate swabs from sexual assault kits contain mostly female epithelial cells with relatively few sperm cells. Differential extraction, the established method of separating these cell types, is labor-intensive, time-consuming and often fails to improve sperm to epithelial cell ratios sufficiently to resolve a minor male DNA profile. This project investigated the use of antibodies specific to proteins expressed on the acrosome of human sperm cells. A cocktail of antibodies specific for acrosome proteins were previously shown to have a high affinity for spermatozoa in pilot flow cytometry studies.

Immunomagnetic beads (IMBs) conjugated with anti-SPAM1, anti-SPACA1 and anti-ZPBP antibodies were constructed and initial experiments showed that the highest quantity of sperm was isolated with anti-SPAM1 at 0.05 mg/mL, anti-SPACA1 at 0.025 mg/mL, or anti-ZPBP at 0.025 mg/mL. Coupling antibodies for each of these proteins individually to magnetic beads was shown to sufficiently isolate sperm for DNA extraction from single donor sperm cell concentrations at  $10^3$  cells/mL. The technique proved to be sensitive with full STR profiles being generated from sperm cell concentrations of  $10^3$  cells/mL using the anti-ZPBP antibodies and mixtures of all three antibodies. When sperm cells were mixed with female vaginal epithelial cells (4:1 female to male), a greater carryover of the female epithelial cell fraction was observed when sperm isolation by IMBs was performed prior to cell lysis compared to when preferential lysis of epithelial cells was performed before sperm isolation.

Initial experiments were performed with Dynabeads® M-270 Carboxylic Acid, but these beads were found to bind both sperm and female vaginal epithelial cells non-specifically. Blocking the beads with bovine serum albumin (BSA) reduced the non-specific binding of both sperm and epithelial cells. Given the reduced amount of isolated sperm and subsequently extracted DNA from the antibody-coupled, blocked magnetic beads, optimization of the cell isolation process requires further exploration to improve sperm selection.

### Immunomagnetic Beads, Spermatozoa, Sexual Assault

## **B9 DNA Methylation of Decedent Blood Samples to Estimate the Chronological Age of Human Remains**

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**Learning Overview:** After attending this presentation, attendees will understand the strengths and current limitations when employing DNA pyrosequencing to predict the donor age of a blood sample.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with the statistical findings of our age prediction models based on the correlation between the methylation profile and chronological age. We will also provide data on the accuracy of the age predictions.

Chronological age estimation may offer valuable investigative leads in human identification cases. Bisulfite pyrosequencing analysis of single CpG sites on five genes (KLF14, ELOVL2, C1orf132, TRIM59, and FHL2) was performed on 264 postmortem blood samples from individuals aged three months to 93 years. The goals were to develop age prediction models based on the correlation between the methylation profile and chronological age and to assess the accuracy of the prediction. Linear regression between methylation levels and age at each CpG site revealed that the five markers show a statistically significant correlation with age.

The methylation data from a training set of 160 postmortem blood samples were used to develop an age prediction model with a correlation coefficient of 0.65, explaining 73.1% of age variation, with a mean absolute deviation from the chronological age of 7.60 years. The accuracy of the model was evaluated with a test set of 72 samples producing a mean absolute deviation of 7.42 years.

The training and test sets were also categorized by specific age groups to assess accuracy and deviation from chronological age. The data for both sets revealed a lower prediction potential as an individual increases in age, particularly for the age categories above 50 years.

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### **DNA Pyrosequencing, Decedent Blood, Donor Age Estimation**

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**B10 An Improved Method for the Analysis of Bisulfite Modified DNA Samples**

*Deborah Silva, PhD\*, Hofstra University, Hempstead, NY; Caitlin Lydon, Hofstra University, Hempstead, NY*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of developing new and improved methods to analyze forensic samples that undergo harsh chemical treatments such as bisulfite modification.

**Impact Statement:** This presentation will impact the forensic science community by presenting a modified protocol that can be used to improve the analysis of samples that are bisulfite modified.

DNA is important evidence in forensic cases and the human genome has been used to identify persons involved in crimes since the 1980s. More recently, forensic analysts have begun to expand the study of DNA and its capabilities to aid in forensic investigations and started to explore the epigenome.

Throughout life, a person is exposed to the external environment and behavioral habits affect specific portions of the DNA. These changes affect gene expression without changing the DNA sequence. The epigenome is the “layer of information” on top of our existing genetic code, and it contributes to physical and behavior differences between individuals and complete human populations.<sup>1</sup>

DNA methylation is the most studied epigenetic modification and different methylation patterns have been shown to be useful to identify body fluids and to estimate the age of a sample donor, for example.<sup>2,3</sup>

When analyzing DNA methylation, bisulfite modification is a process often used to get information about a DNA strand’s methylation pattern since the methylated status of a DNA sample is lost during the process of PCR amplification.

However, the process of treating DNA with sodium bisulfite is harsh since it involves changes in the chemical structure of the DNA (unmethylated cytosines are converted into uracils). This treatment is not ideal to be used with forensic samples since DNA is damaged in the process, but it is necessary to make possible the analysis of methylation patterns.

The goal of this research was to use a modified method to bisulfite treat the DNA followed by a modified PCR to repair the damaged strands and to improve data generation. To achieve this goal, different DNA samples were tested using the modified protocol. These samples included standard DNA, DNA extracted from buccal cells without undergoing any further treatment, and DNA samples extracted from buccal cells that were exposed to UV light and heat treatment to mimic forensic samples.

First, the EZ DNA Methylation-Lightning™ kit was used to bisulfite modify all DNA samples. Then a modified PCR that involved adding repair enzymes to the reaction mix was performed and an extra step to thermocycling conditions was added to repair the damaged DNA before the start of the amplification process. The NanoDrop™ One was used to analyze the results of the modified protocol to confirm the quality and quantity of methylated DNA samples.

The results obtained in this study are important to show that it is possible to improve the quality of DNA samples after going through a harsh chemical modification, which will then provide better results in DNA amplification and other downstream methods and analysis.

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2. Silva, D.S.B.S.; Antunes, J.; Balamurugan, K.; Duncan, G.; Alho, C.S.; McCord, B. Evaluation of DNA methylation markers and their potential to predict human aging. *Electrophoresis*. 2015; 36:1775–1780.
3. Vidaki, A.; Giangasparo, F.; Syndercombe Court, D. Discovery of potential DNA methylation markers for forensic tissue identification using bisulphite pyrosequencing. *Electrophoresis*. 2016; 37(21):2767-2779.

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**Forensic Epigenetics, Damaged DNA, Modified Bisulfite Treatment**

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## **B11 The Use of a Mobile Fingerprint Identification Device in the Field and DNA Evidence: Benefits and Consequences**

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**Learning Overview:** After observing this presentation, attendees will have a better understanding of the feasibility of collecting fingerprints at a death scene in cases where the decedent's hands may contain crucial evidence that needs to be collected and whether the risk of contamination outweighs the benefits that a mobile fingerprint device can provide.

**Impact Statement:** This presentation will impact the forensic science community and the evolving modern forensic science world by providing guidelines of proper handling and decontamination of a mobile fingerprint device when used in the field to expedite the identification and investigation and at the same time to protect the DNA evidence for downstream STR analysis. The evidence of DNA contamination on a mobile fingerprint device may affect the ability to implement its use in the field when using the simple decontamination methods like alcohol wipes etc.

Identifying a decedent is an essential element of death investigation. Mobile fingerprint devices could prove extremely beneficial in identifying decedents in the field so the investigation can begin. The mobile fingerprint identification device used in this study relies on the refractive indexes produced by the valleys and ridges within a person's fingerprint. The light passes through the area sensor of the device and into a lens which then focuses the light, and the camera converts it into a digital image. These devices can typically capture anywhere between 1-10 images which can then be sent to the AFIS database to perform a search of the ten-print database.

This study assessed whether alcohol wipes were sufficient to reduce DNA contamination on the device so that it could be used in the field by investigators for rapid identification. In order to do so, 20 subjects were selected for the study. Each subject was digitally fingerprinted by placing both index fingers on the area sensor of the device. Three swab sets were taken for each test case; the first set of the swab was of the entire device after the device had been handled by the operator, the second set of the swab was only of the sensor area of the device after fingerprinting the subject, and the third set was of the entire device after cleaning it with an alcohol wipe. In addition, a control fingerprint of the right index finger of each subject was taken onto an acetate fingerprint card.

DNA from all sets collected from each subject was extracted using Promega's Maxwell 48 extraction instrument and quantified using Promega's PowerQuant system on a QuantStudio 5. All qualified samples were amplified using Promega's PowerPlex Fusion using a ProFlex Thermal Cycler and amplified DNA was run on a 3500 Genetic Analyzer. The data was analyzed using GeneMapper-IDX software. The DNA obtained from 38 swabs out of 86 total swabs collected did not qualify for STR amplification as per the laboratory autosomal STR amplification guidelines. All the swabs (pre cleaning and post cleaning) collected from the device were successfully amplified with useable profiles. The data from these showed contamination from the operator in majority of the swabs. In some cases, contamination from the subjects was also seen on the device. Only 3 subjects out of 20 could be STR-typed positively from their fingerprints taken on the sensor area of the device. This implies that although these mobile fingerprint devices can rapidly identify decedents in the field and allow the investigation to begin, they pose a risk for contamination which can be harmful to the investigation if there is evidence that needs to be collected from the hands of the decedent.

This research is relevant to modern forensic science because it highlights that alcohol wipes on their own are not sufficient to prevent DNA contamination on mobile fingerprint identification devices and therefore other methods of decontamination and handling of the device should be explored before employing its use in the field by investigators. One such method could be to clean the device with a 10% bleach solution, only handle the device when wearing a mask and gloves. Another method may be to use a new device at each scene.

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### **Mobile, Identification, Contamination**

## B12 Evaluating Different Collection Tools for Transfer DNA Deposited on Wooden Surfaces

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**Learning Overview:** After viewing this presentation, attendees will appreciate how collection tools can impact subsequent genetic testing.

**Impact Statement:** This presentation will impact the forensic science community by providing data that addresses the amount of biological material collected from wood surfaces by two different swabs and how that influences DNA profile composition.

The collection of transfer DNA from evidentiary items is an increasingly common and important part of many criminal investigations. A significant amount of research has been devoted to optimizing the collection and analysis of transfer DNA samples. Collection of transfer DNA typically utilizes a cotton swab, which has been shown to be the best choice for a variety of surfaces.<sup>1,2</sup> However, as DNA extraction and amplification technologies continue to improve the ability to purify and analyze small quantities of degraded DNA, collection techniques should also strive for the maximum collection of biological material from item surfaces and the maximum release of cells during the extraction process. A smaller swab size to concentrate sample collection and a layered swab head to trap more cells might increase the amount of biological material obtained during the collection process in comparison to the traditional cotton tipped swabs utilized by many investigative agencies.

The goal of this study is to compare the results of two different swab types in the collection of transfer DNA to test for differences in the quantity of the DNA collected from the sampled item and the quality of the DNA profile obtained. The two types of swabs utilized for comparison were a traditional cotton-tipped swab with wooden handle (Swab A) (Puritan) and a cotton swab with a layered and ejectable tip from a plastic handle (Swab B) (Gentueri Inc.). Twelve wooden rulers were divided into two even sections and were decontaminated with a 10% bleach solution and exposure to UV light for 15 minutes. Three participants were asked to vigorously handle all surfaces of a ruler for one minute. Immediately after handling, one section of the ruler was wet-swabbed with Swab A, and the other section was wet-swabbed with Swab B. This was repeated on four different days, for a total of 24 samples (12 samples per swab type). The QIAamp DNA Mini Kit (QIAGEN) was used for purification of the DNA. The following Applied Biosystems™ products were used to process the samples through the DNA analysis workflow: samples were quantified with Quantifiler™ Trio DNA Quantification Kit on a 7500 Real-Time PCR System, amplified using Globalfiler™ Amplification Kit on a ProFlex™ PCR System, and analyzed on a 3500xL Genetic Analyzer. The following null hypotheses were tested: (1) the number of alleles detected will not vary between the two swabs and (2) the quantity of DNA collected will not vary between the two swabs.

During collection, it was noted that Swab B was easier to use, and the ejectable tip could help eliminate accidental contamination. The DNA quantity for the samples in this study ranged between 0.0006 ng/ul and 0.0123 ng/ul. No sample yielded alleles at all loci. T-tests were performed to investigate differences in the DNA quantities and number of alleles detected between Swab A and Swab B. A significant difference in DNA quantity ( $p=0.004$ ) and number of alleles ( $p=0.0007$ ) exists between the two swab types. Therefore, both null hypotheses are rejected since Swab A collected more DNA and more alleles were detected during amplification of Swab A samples. In conclusion, while Swab B was much easier to use than Swab A, Swab A performed better in collection and release of biological material from wooden surfaces.

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1. Comte, Jennifer, et al. Touch DNA Collection – Performance of Four Different Swabs. *Forensic Science International: Genetics*, vol. 43, 25 June 2019, p. 102113., doi:10.1016/j.fsigen.2019.06.014.
2. Hedman, Johannes et al. Enhanced Forensic Dna Recovery with Appropriate Swabs and Optimized Swabbing Technique. *Forensic Science International: Genetics*, vol. 53, 12 Mar. 2021, p. 102491., doi:10.1016/j.fsigen.2021.102491.

### Transfer DNA, Cotton Swabs, DNA Recovery

## B13 Extraction Techniques to Increase DNA Recovery From Touch DNA Samples

Ryan James Hinojosa, BS, Cedar Crest College, Allentown, PA; Jillian Conte, PhD\*, MicroGEM, Charlottesville, VA; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA; Janine Kishbaugh, MSFS, Cedar Crest College, Allentown, PA

**Learning Overview:** After attending this presentation, attendees will understand how the method by which DNA is extracted from touch DNA samples greatly impacts DNA yields.

**Impact Statement:** This presentation will impact the forensic science community by advancing the knowledge of how processing multiple cuttings from touch DNA samples in multiple tubes versus a single tube can improve DNA yields. This work could help forensic biologists improve their workflow at the DNA extraction step to increase DNA yield from touch DNA samples.

Increasingly crime laboratories are receiving evidence with touch DNA. In this work, touch DNA is defined as the DNA that is recovered from a sample in which a person's skin has directly or indirectly come into contact with, composed of celled and cell-free DNA.<sup>1,2</sup> One challenge that exists with recovering touch DNA from evidence is that it is not visible to the naked eye, thus locating a sample can be difficult and DNA analysts often must resort to making an assumption as to how the item was handled. Samples are then collected by swabbing, cutting, scraping, or vacuuming. In this research, sample collection was conducted by cutting and vacuuming.

Following visualization of touch DNA by a novel fluorescent detection method, three 0.5 cm<sup>2</sup> cuttings were taken from white cotton shirts that were grabbed by volunteers. The "standard method" consists of the three cuttings placed into one 2.0 mL microcentrifuge tube and extraction being conducted. The "combination method" consists of each of the three cuttings placed into separate microcentrifuge tubes and a portion of the extraction process being conducted and then combining these partial extracts into one tube to complete the extraction. Two extraction methods were used and compared – a solid phase extraction that utilizes columns and wash steps (QIAGEN) and a temperature driven, enzymatic extraction (MicroGEM). Samples were quantified for comparison of DNA recovery. Student t-tests and ANOVA statistics were conducted (with  $p \leq 0.05$  as the level of statistical significance). The combination method for sample processing obtained up to four times more DNA in some donors. The minimum amount of DNA obtained was about 11 times higher with the combination method than the standard method. This research furthers the knowledge of the forensic community and helps forensic biologists recover higher DNA quantities through sample processing methods and extraction approaches.

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1. Burrill, J.; Rammenou, E.; Alawar, F.; Daniel, B.; Frascione, N. Corneocyte lysis and fragmented DNA considerations for the cellular component of forensic touch DNA. *Forensic Science International: Genetics* 2021;51:102428. <https://doi.org/10.1016/j.fsigen.2020.102428>.
2. Burrill, J.; Daniel, B.; Frascione, N. A review of trace "Touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics* 2019;39:8–18. <https://doi.org/10.1016/j.fsigen.2018.11.019>.

### Touch DNA, DNA Extraction, Sample Collection



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## B14 Modeling Assault From Touch DNA

*Jamie Fredericks, PhD, Eastern Kentucky University, Richmond, KY; Michael Lane, PhD, Eastern Kentucky University, Richmond, KY; Beighley Ayers, \* Eastern Kentucky University, Richmond, KY*

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**Learning Overview:** Attendees can expect to learn: (1) There is no correlation between the concentration of DNA collected and the average force applied (how hard the target was hit) or the contact time (the time of skin-to-skin contact between the participant's fist and target) of single strikes, and (2) It is possible to determine the sequence of participation when two participants strike a skin surface (one after each other).

**Impact Statement:** Our study demonstrates that touch DNA has the potential to determine the sequence of punches when a victim is subjected to multiple strikes by multiple individuals. This information can play a pivotal role in determining and corroborating eyewitness statements and establishing who struck a victim of assault and when.

Advances in DNA analysis have led to the ability to generate a DNA profile from minute amounts of DNA, such as 'touch' DNA. Touch DNA is biological material left behind when an individual has handled an object. Forensic medical examiners are often required to collect biological material from individuals that claim to have been assaulted and is playing an increasing role in solving crimes. Traditionally, when an assault has occurred, DNA is collected from hands or fingerprints; however, any area of skin that has made skin to skin contact between the victim and the alleged perpetrator could be swabbed. This aim of this study was to determine whether the forces applied during a mimicked aggravated assault scenario could be modeled from the DNA collected. We investigated the nature of touch DNA transfer, including DNA concentration and subsequent profile generated, when different measures of force are applied and multiple 'assailants' made skin to skin contact with a 'victim'. We wanted to determine whether primary and secondary DNA transfer could aid investigators in determining the sequence of events.

A novel apparatus was developed to mimic aggravated assault. A padded synthetic skin was placed on top of a force plate that would then be punched by participants. After each strike, the skin pad was swabbed, and the DNA concentration was determined before a DNA profile (using the PowerPlex Fusion kit) was generated. The force plate measures various parameters of the strike including, peak force, mean force, and time of force application. Each participant would strike the force plate once, either as an isolated event or as a combination with an additional individual.

Preliminary results have shown that although there was no correlation between the concentration of DNA collected and the average force applied (how hard the target was hit) or the contact time (the time of skin-to-skin contact between the participant's fist and target) of single strikes, it was possible to determine the sequence of participation when two participants strike the skin pad (one after each other). In 89 % of the samples, the second individual that punched the skin pad was observed to be the major contributor.

This study demonstrates that touch DNA has the potential to determine the sequence of punches when a victim is subjected to multiple strikes by multiple individuals. This information can play a pivotal role in corroborating eyewitness statements and establishing the 'by who and when' sequence of events.

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### Assault, Touch DNA, Force

**B15 The Persistence of Transfer DNA on Touched Objects Over Time**

*Alba Craig, BA\*, University of Indianapolis, Indianapolis, IN; Krista Latham, PhD, University of Indianapolis, Indianapolis, IN; Cynthia Cale, MS, Houston Forensic Science Center, Houston, TX; Stephen Nawrocki, PhD, University of Indianapolis, Indianapolis, IN*

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**Learning Overview:** The goals for this presentation include attendee appreciation for and increased understanding of: (1) the complexities of transfer DNA sampling and interpretation, (2) the effects of long-term storage on the quantity and quality of transfer DNA recovered from potential evidence items, and (3) how sampling location and timing can influence the DNA profile and interpretation.

**Impact Statement:** This presentation will impact the forensic science community by increasing the understanding of the persistence of DNA obtained from touched objects stored in a laboratory setting, by providing information on potential changes in DNA quantity and the quality of resulting DNA profiles over time. This will, in turn, provide information that may prove meaningful in terms of the urgency of processing DNA evidence in forensic laboratories experiencing backlogs.

The collection, analysis and interpretation of transfer DNA has been and continues to be highly studied in the forensic DNA community. However, there is a lack of studies that investigate the impact of long-term storage on the persistence of transfer DNA over time. For this study, 12-inch wooden rulers, divided into 9 equal parts for sampling, were handled by participants in order to monitor potential changes in the quantity of transfer DNA obtained and the quality of the resulting DNA profiles over time. Each participant was assigned one decontaminated ruler to handle for a two-minute period. Participants did not wash their hands prior to handling and were instructed to evenly contact the entire ruler surface. Using a single wet swab, one sample was collected from each section of each ruler every other week for fifteen consecutive weeks. To mimic evidence storage in forensic laboratories, the rulers were stored at room temperature in separate paper bags sealed with tape after each sampling event. The following null hypotheses were tested: (1) there will be no change in DNA quantity over time, (2) there will be no change in the quality of DNA profiles over time, and (3) no foreign DNA will be detected.

Samples were extracted using the QIAamp DNA Mini Kit (QIAGEN). Samples were further processed through DNA analysis workflow utilizing Applied Biosystems™ products: samples were quantified with Quantifiler™ Trio DNA Quantification Kit on a 7500 Real-Time PCR System; samples were amplified using Globalfiler™ Amplification Kit on a ProFlex™ PCR System and analyzed on a 3500xL Genetic Analyzer. Additionally, likelihood ratios were generated using STRmix™ v2.6.1.

The data collected in this longitudinal study provides insight into the effects of long-term storage on the recovery of DNA from touched objects. DNA quantity and DNA profile quality varied over time and between participants with some samples yielding no DNA profiles. Likelihood ratios ranged from uninformative to very strong support in favor of inclusion. Interestingly, one sample resulted in the exclusion of the ruler handler. Yet in another sample, a false exclusion of the ruler handler occurred because the apparent single-source profile was most likely a mixed DNA profile.

Based on the results obtained from this study, all null hypotheses were rejected. In conclusion, long-term storage of DNA evidence can have an influence on the persistence of transfer DNA over time which, in turn, can have an effect on forensic investigations.

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**Transfer DNA, STRmix™, DNA Persistence**

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## **B16 An Evaluation of Collection Methods for Direct Polymerase Chain Reaction (PCR) Amplification of Touch DNA Samples**

*Anna Salmonsens, MFS\* Bode Technology, Lorton, VA; Abigail Bathrick, MFS, Bode Technology, Lorton, VA; Jonathan Davoren, MS, Bode Technology, Lorton, VA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of direct polymerase chain reaction (PCR)-compatible collection methods and the use of direct PCR for touch DNA samples.

**Impact Statement:** This presentation will impact the forensic science community by recommending effective methods for collecting touch DNA from different substrates prior to direct PCR and presenting data comparing direct PCR and standard processing of touch DNA samples.

Improved methods for generating high-quality DNA profiles from touch DNA samples are of considerable interest to forensic DNA laboratories. Direct PCR amplification, a sample processing method in which a swab or substrate punch is added directly to an amplification reaction without prior extraction or quantification, has been identified as a method that may improve DNA profiles from low-yield touch DNA evidence samples. Direct PCR maximizes the amount of DNA template in a reaction by eliminating the DNA loss that occurs during DNA extraction, quantification, and concentration; however, the amount of DNA available for direct PCR can be affected by the method used for DNA sample collection. This study aimed to identify collection methods that maximize DNA recovery from common touch DNA substrates and to determine whether direct PCR or standard processing methods result in higher quality DNA profiles.

Nine direct PCR-compatible collection methods were used to collect touch DNA from cotton denim, wool, and polyester fabric; plastic microscope slides; metal tools; handgun grips; vinyl shutter samples; brass cartridge casings; foam cups; concrete bricks; and unfinished wooden tool handles. Collection was performed with Puritan® cotton swabs, Copan microFLOQ® direct swabs, and Whatman non-indicating FTA paper that were moistened with sterile water, moistened with 0.1% Triton™ X-100, or left dry. The fabric substrates were sampled via cutting. For each collection method, processing method, and substrate type, eight replicates were prepared from three donors. Samples were processed with two methods: 1) standard processing with DNA extraction and quantification and 2) direct PCR. Amplification for both the extracted and direct PCR samples was performed with the GlobalFiler™ PCR Amplification kit.

Direct PCR was successful for samples collected from plastic slides, polyester fabric, metal tools, handgun grips, vinyl shutters, foam cups, and unfinished wooden tool handles. The highest quality direct PCR results were obtained using microFLOQ swabs for collection. Direct PCR and standard processing produced generally comparable DNA profile results. Direct PCR of samples collected from metal tools and wooden tool handles with dry microFLOQ swabs and handgun grips with microFLOQ swab & water resulted in significantly higher quality DNA profiles than standard processing. Direct PCR was unsuccessful for samples collected from concrete bricks; cartridge casings; and denim, wool blend, and 100% wool fabrics, regardless of the method used for collection.

These results indicate that the success of direct PCR is highly dependent on the substrate from which samples are collected, whereas profile quality is affected by the efficacy of the collection method. In conclusion, direct PCR is an effective method for processing evidentiary touch DNA samples collected from certain substrates, and microFLOQ swabs were the most effective collection method for direct PCR of those tested.

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### **Direct PCR, Touch DNA, Evidence Collection**

## B17 A Combined Extraction of Protein and DNA From Touch Evidence on Ammunition Cartridge Cases

Ashlee Lynch, BS\*, University of California-Davis, Davis, CA; Glendon Parker, DPhil, University of California-Davis, Davis, CA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the options for retrieving touch evidence from ammunition bullet cases.

**Impact Statement:** This presentation will impact the forensic science community by presenting an alternative technique that helps to maximize biological information retrieved from challenging touch evidence.

DNA analysis of touch evidence on cartridge cases has proven difficult despite numerous attempts to improve collection and extraction methods for this important type of evidence. Numerous factors influence the variability of touch evidence such as shedder status, pressure and duration of contact, surface type, and collection and storage.<sup>1,2</sup> Retrieval of biological information from cartridge cases are even more challenging since firing abrades the surface of the case, removing material, and metal in the case can react with DNA, resulting in oxidation and cleaving of the DNA backbone.<sup>3,4</sup> Protein is more robust chemically than DNA, and contains genetic and contextual information that could be useful to investigators.<sup>5</sup> Extraction and analysis of DNA and proteins from touch evidence can provide the maximum amount of information needed for probative results, especially when DNA is limited.<sup>6</sup>

This study evaluated the efficiency of transferring artificial DNA and protein from a glass microscope slide surface into the workflow for seven different collection and transfer methods: the standard wet-dry cotton swab was compared with alternative methods, a 'Copan microFLOQ® direct' swab, with and without lysing agent, and with and without extraction buffer, a cell scraper, and adhesive silicone gel-film sheets.

The results obtained determined that overall the 'Copan microFLOQ® direct' was the most efficient and consistent method with an average transfer of  $58 \pm 22\%$  efficiency for DNA and  $55 \pm 32\%$  for protein compared to  $27 \pm 24\%$  and  $2 \pm 27\%$  when using cotton swabs respectively. The evaluation was taken a step further to measure transferred DNA and protein from real fingerprints on a glass microscope slide. Results determined that the cotton swab and the 'Copan microFLOQ® direct' collected an average of  $11.8 \pm 20$  ng and  $15.7 \pm 18$  ng of total DNA, respectively, and  $1200 \pm 1200$  RDU and  $2300 \pm 1600$  RDU of total relative protein density, respectively. This level of relative variation is high but consistent with previous studies for fingerprint deposition.<sup>4</sup> When normalized for individuals the 'Copan microFLOQ® direct' increased performance by 1.7-fold ( $p=0.23$ ) for DNA and 1.9-fold ( $p=0.02$ ) for protein.

Finally, real-life scenarios were mimicked where the cotton swab and 'Copan microFLOQ® direct' were used to transfer DNA and protein from unfired and fired cartridge cases into the workflow. The cotton swab and the 'Copan microFLOQ® direct' collected an average of  $1.1 \pm 1.5$  ng and  $2.8 \pm 2.4$  ng of total DNA, respectively, and  $660 \pm 380$  RDU and  $1200 \pm 1500$  RDU of total relative protein density, respectively for unfired cases. When fired there was a significant reduction in the amount of DNA material. The cotton swab and the 'Copan microFLOQ® direct' collected an average of  $0.3 \pm 0.5$  ng and  $0.5 \pm 0.6$  ng of total DNA, respectively, and  $400 \pm 340$  RDU and  $530 \pm 450$  RDU of total relative protein density. When normalized for individuals the 'Copan microFLOQ® direct' increased yields by 3.2-fold ( $p=0.03$ ) for DNA and 2.0-fold ( $p=0.06$ ) for protein when unfired and 3.7-fold ( $p=0.09$ ) for DNA and 1.6-fold ( $p=0.24$ ) for protein when fired. The firing process was harsher on DNA than on protein. Protein was 2.2-fold ( $p=0.07$ ) more persistent than DNA on cartridge cases after firing.

We conclude that transfer of residual biological material from fingerprints is improved when using 'Copan microFLOQ® direct' swabs compared to the wet-dry cotton swab method, and that the improvement applies to both idealized surfaces such as microscope slides, and inadequate surfaces such as unfired and fired cartridge cases. We also observe that protein is more stable during the firing process and potentially can provide additional identifying and contextual information for investigators for this difficult type of evidence.

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### Touch Evidence, Cartridge Cases, Biological Material

### **B18 Can Variations in DNA Profile Composition Collected From Different Areas of the Same Handgun Inform Future Sampling Strategies?**

*Cynthia Cale, MS\*, Houston Forensic Science Center, Houston, TX; Jessica Miller, MS, Marion County Coroner's Office, Indianapolis, IN; Krista Latham, PhD, University of Indianapolis, Indianapolis, IN; Erica Cantor, MS, Indiana University School of Medicine, Department of Hematology and Oncology, Indianapolis, IN*

**Learning Overview:** After attending this presentation, viewers will be introduced to variations in DNA profile composition and statistical interpretation via likelihood ratios (LRs) calculated from samples collected in different locations of the same single-owner firearm.

**Impact Statement:** This presentation will impact the forensic science community by informing practitioners as to which specific area(s) of a handgun may be best suited for the recovery of DNA and the generation of informative profiles.

Handguns are a common piece of evidence associated with crimes and criminal activity. This study was designed to systematically test whether there is a detectable difference in the quality and quantity of DNA obtained from four locations (grip, trigger, slide, and magazine) sampled from a single-owner firearm that had been cleaned and briefly handled. Eleven handguns owned by eleven different law enforcement personnel were used in this study. A questionnaire was completed by each participant, which discerned length of ownership, length of daily contact, frequency of cleaning, time since last firing, and accessibility by other individuals. Each firearm was unloaded, wiped with a 10% bleach solution, reloaded, and handled in a way to simulate firing by the owner. Samples from the four designated areas of the firearm were collected separately using a single-swab technique. Each swab was moistened with 100  $\mu$ L of sterile PBS before swabbing.

A total of 44 samples were extracted utilizing the QIAamp® DNA Mini Kit per manufacturer's instructions. Extracted DNA was amplified with the GlobalFiler™ PCR Amplification Kit and analyzed on an AB 3130xl genetic analyzer. STRmix™ v2.6.1 was utilized to aid in data interpretation. Deconvolution of any mixtures was completed before comparisons to known reference samples from the owners. The NIST1036 database was used to calculate LRs. The SWGDAM scale of verbal qualifiers was used to express the degree of support for a specified proposition (H1 – owner of gun is a contributor) relative to an alternative proposition (H2 – unknown, unrelated individual is a contributor).

Data were obtained from all 44 samples. The average amount of DNA obtained from each area decreased in the following order: grip, magazine, slide, trigger. Single-source DNA profiles (36% grip, 27% magazine, 55% slide, 27% trigger) and mixed DNA profiles (64% grip, 73% magazine, 45% slide, 73% trigger) were observed. There was no discernable pattern as to the number of contributors based on swab area. The majority of the LRs for all samples provided very strong support ( $LR \geq 1,000,000$ ) in favor of H1. For Gun 5, the LR for the magazine sample provided limited support in favor of H2 ( $0.5 \geq LR \geq 0.01$ ) while the LRs for the slide and trigger samples provided moderate support ( $100 \leq LR \leq 9,999$ ) and strong support ( $10,000 \leq LR \leq 999,999$ ) in favor of H1, respectively. Likewise, the LRs for the trigger samples from Guns 4 and 6 provided moderate support ( $100 \leq LR \leq 9,999$ ) in favor of H1, while the LR for the trigger sample from Gun 8 provided strong support ( $10,000 \leq LR \leq 999,999$ ) in favor of H1.

The results of this study demonstrate the complexity of DNA transfer. The detection of mixtures in the majority of the samples obtained from cleaned weapons suggests either "cleaning" of handguns is not effective or extraneous DNA is easily transferred via the owners' hands. Regardless, the LRs for most samples strongly support inclusion of the firearm owner known to have touched the handgun. However, LRs do vary within the samples collected for a single handgun suggesting some areas of a firearm might be more informative in the interpretation of relationships between DNA evidence and criminal acts.

#### **Transfer DNA, Handguns, Probabilistic Genotyping**

## **B19 The Quantification of Metal Ions Recovered During DNA Analysis of Brass Ammunition Using Inductively Coupled Plasma/Optical Emission Spectrometer (ICP/OES)**

*Natalia Czado, MSFS\*, Sam Houston State University, Huntsville, TX; Sheree Hughes, PhD, Sam Houston State University, Huntsville, TX*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the quantitative presence of copper and other metal ions during the recovery and analysis of touch DNA deposited on brass ammunition.

**Impact Statement:** This presentation will impact the forensic science community by informing them on the expected quantity of metal ions recovered during commonly used collection, lysis, and purification methods for processing DNA deposited on brass ammunition and providing guidance on improving downstream genotyping.

It is often challenging to obtain Short Tandem Repeat (STR) profiles from DNA recovered from the surface of ammunition, especially those comprising copper alloys. This problem is not only due to the low amount of DNA deposited, but also metal ions causing degradation and damage by interacting with the DNA, and/or inhibition during the polymerase chain reaction (PCR). Numerous studies have examined collection and extraction methods aimed at increasing genotyping success from these challenging samples.<sup>1,2</sup> However, results have been inconsistent. Although there is consensus that metal ions, especially copper, can intercalate with DNA or cause PCR inhibition, to date, no study has quantified the amount of metal ions recovered during the collection of touch DNA on brass ammunition and that may persist after DNA purification.<sup>3-5</sup>

This study examines the presence and quantity of copper and zinc ions during DNA collection and purification and their possible effects on genotyping success. The effectiveness of various DNA collection and purification methods commonly used by forensic laboratories to process brass ammunition for DNA evidence was also investigated. The amount of copper, zinc, and other metals co-recovered from fired and unfired brass casings during DNA collection (using numerous soaking, swabbing, and direct PCR protocols) was quantified via Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). This same panel of metals was subsequently quantified after DNA lysis and purification.

As expected, the largest quantities of metal ions recovered with all collection methods were copper and zinc, for both fired and unfired casings. Although some differences were observed between the various DNA collection and purification methods tested, the quantity of metal ions were significantly reduced prior to STR amplification. Therefore, the results suggest that additional purification to remove more metal ions may not be necessary for increased genotyping success, but rather using alternative markers such as single nucleotide polymorphisms (SNPs) with next generation sequencing (NGS) technologies to examine the low amounts of damaged and degraded DNA.

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### **Copper, Touch DNA, Brass Ammunition**

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## **B20 Validation of Lateral-Flow Immunoassay Test for Rapid Screening of Cocaine Samples**

*Corey Nida, PhD\*, Drug Enforcement Administration, Dulles, VA; Hunter Haddad, BS, Drug Enforcement Administration, Dulles, VA; Sandra Rodriguez-Cruz, PhD, Drug Enforcement Administration, Dulles, VA*

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**Learning Overview:** After reviewing this poster, attendees will have a better understanding of the procedures used to complete an in-house validation of two commercial lateral-flow immunoassay (LFIA) tests for use during the analysis of suspected cocaine submissions.

**Impact Statement:** This poster will impact the forensic science community by providing information on how LFIA tests can be used as a rapid screening tool for samples containing cocaine and determine its fitness for purpose within the seized-drug laboratory analysis workflow.

The in-house validation requirements and results of two commercially available cocaine LFIA tests (Rapid Response™ Single Drug Test Strips [BTNX, Inc.] and Premier Identification Kits [Premier Biotech, Inc.]) are presented. Three primary characteristics were assessed to investigate the utility of the above-mentioned cocaine LFIA tests: selectivity, limit of detection (LOD), and repeatability (short-term precision).

Selectivity was evaluated by testing individual compounds and mixtures, in triplicate. In addition to the target analytes (cocaine hydrochloride and cocaine base) and other routine controlled substances, several of the most commonly encountered adulterants, diluents, and alkaloids in cocaine samples were individually tested. False-positives were observed with tropacocaine and benzoylecgonine for one of the cocaine-LFIA tests, while only benzoylecgonine was observed as a false positive in the other.

Mixtures containing varying amounts of (5%, 50%, 95%) cocaine hydrochloride or cocaine base with lactose, mannitol, levamisole, or phenacetin were also included as part of the scope of the selectivity. False-negatives were observed in 13 of the 72 prepared mixtures for one of the cocaine-LFIA tests, while no false-negatives were observed in the other.

LOD was evaluated by analyzing five replicates of cocaine hydrochloride and cocaine base reference materials at decreasing concentrations until at least one negative result was observed amongst the replicates. The experimentally determined LOD for one of the commercially available cocaine-LFIA tests was 1 milligram (mg), while that for the other was 2.6 microgram ( $\mu\text{g}$ ).

Repeatability was assessed by performing thirty replicate tests of cocaine hydrochloride, cocaine base, levamisole, and phenacetin. Two separate lots of the commercially available cocaine LFIA tests were included in the scope of this study and no unexpected results were observed for the four compounds tested.

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### **Validation, Cocaine, Immunoassay**

## B21 The Extraction and Identification of Illicit Compounds from Baked Goods Using Paper Spray Ionization Tandem Mass Spectrometry

Isabella Haberstock, BS\*, Duquesne University, Pittsburgh, PA

**Learning Overview:** Attendees will learn about how the QuEChERS solid phase extraction method can be used to extract an illicit compound from a complex matrix containing proteins, carbohydrates, and lipids. Attendees will also learn about the versatility of paper spray ionization mass spectrometry in the rapid analysis of the QuEChERS supernatant for the presence of the illicit compound.

**Impact Statement:** Complex matrices like cookies and other baked goods have become common methods of consumption for illicit substances, and a rapid and accurate method for the extraction and detection of these substances is necessary for ongoing forensic investigations. Forensic toxicological analysis in drug-related cases often involve the detection of substances like MDMA, amphetamine, and methamphetamine, so an efficient solid phase extraction method would benefit time-sensitive investigations.<sup>1</sup> The paper spray ionization method can also be optimized and has the potential to advance the field of drug detection because of its versatility and rapidity.<sup>2</sup>

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The goal of this project is to couple the QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction method with paper spray ionization mass spectrometry to create an optimized extraction and detection method for illicit substances in baked goods. The QuEChERS method, although not typically used for baked goods, isolates the illicit compound from a complex matrix of carbohydrates, lipids, and proteins, allowing for accurate detection of the compound.<sup>3</sup> Paper spray ionization is a versatile method that produces accurate quantitative and qualitative data for drug detection studies.<sup>4</sup> Dough for individual sugar cookies was made using a basic recipe, and the cookies were spiked with 0 $\mu$ L, 1 $\mu$ L, 3 $\mu$ L, 5 $\mu$ L, and 10 $\mu$ L of phenethylamine. Each cookie was extracted in acetonitrile, magnesium sulfate, sodium chloride, and a lipid sorbent for the QuEChERS method.<sup>3</sup> The supernatant was analyzed using the ThermoFisher LTQ-XL for the presence of the phenethylamine peak, which was confirmed through the use of tandem mass spectrometry and collision induced dissociation (CID). Using paper spray ionization mass spectrometry, the phenethylamine peak was seen in the 1 $\mu$ L, 3 $\mu$ L, 5 $\mu$ L, and 10 $\mu$ L phenethylamine cookies. CID on the phenethylamine peak confirmed these results for each cookie. The cookie with 0 $\mu$ L phenethylamine did not display the phenethylamine peak, which confirmed that the cookies were not cross contaminated. Because phenethylamine was successfully extracted and detected using QuEChERS and paper spray ionization, future experiments include the quantification of the illicit substance, the use of MDMA in place of phenethylamine, and the use of the LC-QQQ-MS to further perform targeted analysis on the extracted illicit substance.

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### Mass Spectrometry, QuEChERS, Baked Edibles



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## **B22 A Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method Development and Validation for the Quantitation of Cannabinoids in Hemp and Marijuana Samples**

*Andrea Yarberry, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Jerome Mulloor, MS, National Institute of Standards and Technology, Gaithersburg, MD; Melissa Phillips, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Aaron Urbas, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Walter Wilson PhD, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending this presentation, attendees will learn about LC-MS/MS method development, validation, and application for cannabinoid quantitation.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into LC-MS/MS method development and validation focused on the separation of cannabinoids for quantitation in marijuana and hemp samples.

Cannabinoids are a class of terpenophenolics found in *C. sativa* L. (cannabis), with cannabidiol (CBD) and  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) being the most studied compounds in the class. Prior to the Farm Bill (2018), cannabis was considered a Schedule I controlled substance under the Controlled Substances Act (CSA) and forensics laboratories were only required to determine presence/absence of  $\Delta^9$ -THC in a seized cannabis sample to determine legality. The Farm Bill amended the CSA, differentiating hemp from marijuana. Hemp is now defined as cannabis with a decarboxylated-THC (total THC) content less than or equal to 0.3 % by dry weight and marijuana is cannabis with a total THC content greater than 0.3 % by dry weight. Total THC considers the conversion of  $\Delta^9$ -tetrahydrocannabinolic acid ( $\Delta^9$ -THCA) into  $\Delta^9$ -THC through the heating of the cannabis sample or by using a calculation to mathematically convert the  $\Delta^9$ -THCA to  $\Delta^9$ -THC. While hemp is legal, marijuana remains a controlled substance, which places a new requirement on forensics labs to quantify the amount of total THC in seized cannabis samples. The requirement for total THC quantitation in forensic laboratories, along with the increase in consumer products containing therapeutic cannabinoids has created a need for a robust and selective method for quantitation of cannabinoids in cannabis extracts, oils, edibles, etc.

LC-MS/MS method development investigated selectivity using column chemistry and solvent choice for baseline resolution of the isomeric cannabinoids, where they were indistinguishable using the mass spec. Two C18 columns and three organic solvent mobile phases (B) were considered during method development. Separation using the three organic solvents (0.1 % formic acid in methanol (MeOH), 0.1 % formic acid in acetonitrile (ACN), and 0.1 % formic acid in 50:50 ACN:MeOH by volume) was optimized under isocratic and gradient conditions. The final method utilized a Raptor ARC-18 column (50 mm x 2.1 mm, 2.7  $\mu$ m particle diameter) with gradient separation using mobile phase A: 0.1 % formic acid in water and B: 0.1% formic acid in 50:50 ACN:MeOH. Method validation involved the measurements of up to 17 cannabinoids, total CBD, and total THC in hemp and marijuana plant samples. Reference materials were obtained from the University of Kentucky Proficiency Testing Program and NRC Canada. Cannabinoids were extracted from 500 mg samples via liquid-solid extraction using MeOH. In-house hemp and marijuana samples were analyzed as part of the Cannabis Quality Assurance Program (CannaQAP) at the National Institute of Standards and Technology. This poster will summarize the method development and validation results and highlight some of the challenges that occurred when transferring the method to a state crime laboratory.

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### **Chromatography, Cannabis, Mass Spectrometry**

### **B23 A Novel Approach for Quantitation of Total Tetrahydrocannabinol (THC) in *Cannabis* Plant Extracts by Isotope Dilution Gas Chromatography/Mass Spectrometry (GC/MS)**

*Jerome Mullor, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Melissa Phillips, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Aaron Urbas, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Walter Wilson, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Andrea Yarberr, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

**Learning Overview:** After attending this presentation, attendees will learn about a new approach to quantifying total THC in *Cannabis* plant extracts using isotope dilution GC-MS.

**Impact Statement:** This presentation will impact the forensic science community by providing a novel quantitative GC-MS approach that does not require derivatization and instead utilizes deuterated internal standards to account for the decarboxylation of THCA to  $\Delta^9$ -THC.

Marijuana has been considered a Schedule I controlled substance since the 1970s. Forensic laboratories must evaluate the legality of seized evidence by the content of delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC), the primary psychoactive substance in the *Cannabis* plant. Traditionally, qualitative methods were used to examine seized *Cannabis* plant material. However, the 2018 Farm Bill removed hemp from the controlled substances list and defined it as *Cannabis* containing less than 0.3 % decarboxylated  $\Delta^9$ -THC (total THC) content. The new threshold for differentiating between legal hemp and illegal marijuana implored forensic laboratories to implement new quantitative methods for total THC determination in *Cannabis* plant samples.

Forensic laboratories typically utilize liquid (LC) or gas (GC) chromatography with mass spectrometry (MS) detection as the “gold standard” techniques for quantifying illicit drugs of abuse. GC is usually favored over LC for its shorter separation times and use of carrier gas rather than solvents. Unfortunately, quantitation of *Cannabis* samples by GC is complicated by the decarboxylation of the acidic cannabinoids to their neutral form in the heated zones of the GC. The heat-induced conversion of tetrahydrocannabinolic acid (THCA) to  $\Delta^9$ -THC is incomplete and inconsistent since it depends on the type of liner used and the inlet temperature. Sample derivatization can remedy this issue, but it is time consuming and not feasible for most forensic laboratories.

In this research, a novel isotope dilution (ID) GC-MS method was developed for quantitation of total THC in sample extracts of *Cannabis* (hemp and marijuana) plant samples. The method accounts for the decarboxylation in the inlet by using two deuterated internal standards for THCA and THC (THCA- $d_3$  and THC- $d_3$ ) that have identical chromatographic properties but are differentiable by their mass spectra. The internal standards are added to the sample extracts and calibration standards prior to injection at a 1:1 ratio and a total THC concentration of 0.3 % to mimic a plant extract at the legal threshold. Previously, a GC method was developed to separate nine neutral cannabinoids in under ten minutes using a 35% phenyl column. This method was converted into a selected ion monitoring (SIM) method for total THC utilizing the most abundant ions for  $\Delta^9$ -THC and  $\Delta^9$ -THC- $d_3$ .

Quantitation was performed by extracting the ions for the analyte ( $m/z$  299) and the internal standard ( $m/z$  302) and plotting the mass ratio of total THC to total THC- $d_3$  in the samples. The quantitative ID-GC-MS method was evaluated using several *Cannabis* plant samples and compared with LC with photodiode array detection (LC-PDA) since LC can separately quantify THCA and THC without decarboxylation concerns. Additionally, the limits of detection and quantitation for total THC on the ID-GC-MS method were evaluated. The ID-GC-MS total THC values were in good agreement with the LC-PDA values, demonstrating its potential for use as a quantitative method.

#### **Cannabis, Gas Chromatography, Mass Spectrometry**

## B24 The Classification of Cannabis Varieties Through Headspace Chemical Analysis and Transfer Learning

*Ting-Yu Huang, MS\**, Sam Houston State University, Huntsville, TX; *Jorn Chi-Chung Yu, PhD*, Sam Houston State University, Huntsville, TX

**Learning Overview:** The study provides attendees with an intelligent and rapid solution for cannabis classification by using headspace chemical analysis. A new idea of transforming GC/MS data into image representation to facilitate transfer learning will also be introduced.

**Impact Statement:** The impact of this study is to introduce a transfer learning technique for GC/MS data analysis. A new AI (artificial intelligence) powered framework for classifying cannabis evidence using headspace chemical analysis will be demonstrated.

*Cannabis sativa L.* (cannabis) has been known to produce cannabinoids that may have diverse bioactivities. The Agriculture Improvement Act of 2018 provided a new statutory definition of hemp, which defines the cannabis plant, and any part of it, with a delta-9-tetrahydrocannabinol (THC) concentration of not more than 0.3% on a dry weight basis. Therefore, the new law limits the definition of marijuana only to include cannabis containing more than 0.3% THC. While hemp is used in manufacturing versatile products, marijuana remains a Schedule I controlled substance under the federal Controlled Substances Act. To discriminate between hemp and marijuana, proficient analytical workflows for cannabis samples are essential in forensic laboratories.

Gas chromatography and mass spectrometry (GC/MS) is a commonly accepted analytical instrumentation to identify cannabinoids. However, due to the heterogeneity of cannabis samples, extensive sample preparation is required before instrumental analysis. To increase the throughput, we proposed a headspace sampling approach to extract cannabinoids before using GC/MS for chemical identification. Additionally, the interpretation of GC/MS data typically involves an analyst's operation. Considering the demands of marijuana analysis in forensic laboratories, an automated system to intelligently classify cannabis varieties can increase the throughput and decrease the turnaround time.

Deep learning (DL) model construction is an emerging AI technique to distinguish images for solving classification problems. Transfer learning is a more efficient learning approach and achieves higher performance than training a new DL model. This study aims to explore the transformation of GC/MS data into images to facilitate transfer learning. This approach was applied to construct an intelligent system for differentiating hemp from marijuana. In this study, 228 GC/MS data were collected from 14 standard cannabis varieties with known THC levels. Two varieties (30 data) were hemp, whereas twelve varieties (198 data) were marijuana. The data were resampled into 896x1471x1 arrays. The retention time region of cannabinoids was transformed into pseudo-color heat maps representing sample headspace cannabinoids signature. GoogLeNet, a pre-trained DL model, was retrained by using the standard dataset. Training parameters, including maximum epoch, learning rate, and mini batch size, were discussed.

The results indicated that the classifier could achieve 100% accuracy in classifying hemp and marijuana. The study demonstrates the feasibility of classifying cannabis varieties by training an AI model without collecting a large reference dataset. The probability-based classification outcome given by the proposed workflow also aids in statistical evaluations for interpreting forensic evidence. Moreover, pre-selection of cannabis botanical structures before GC/MS analysis are not required. Overall, combining image processing schemes and transfer learning offers great potential for forensic analysis of cannabis evidence. Our next step is to collect more comprehensive cannabis sources to cover more varieties in the dataset.

### Cannabis, Transfer Learning, Pseudo-Color Heat Maps

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**B25 A Genetic and Chemical Characterization of the Cannabis Products Seized at the Croatian Cannabidiol (CBD) Stores**

*Slavena Cukrov-Bezbradica, PhD\*, Forensic Science Centre, Ivan Vučetić Ministry of the Interior, Zagreb, Croatia; Lucija Barbarić, PhD, Forensic Science Centre, Ivan Vučetić Ministry of the Interior, Zagreb, Croatia*

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**Learning Overview:** Attendees will have an overview of the genetic and chemical data obtained from Cannabis product commercially sold in Croatia.

**Impact Statement:** This presentation will impact the forensic science community by presenting the challenges in differentiation of drug and hemp varieties in Cannabis seizures.

*Cannabis sativa L.* is the most commonly seized illicit drug in Croatia. Recent lifting of restrictions has led to a new problem for the law enforcement: prevention of illegal production and sale in a rapidly advancing cannabis industry that offers a wide range of commercial products. Croatian law allows production of cannabis plant varieties listed in the European Union Plant Variety Catalogue (EU PVC) with less than 0.2% w/w (weight/weight) of total  $\Delta^9$ -tetrahydrocannabinol (THC) content in the dry plant material. Hence, there is a particular need to study the commercial cannabis products. The aim of this study is to assess the genetic and chemical analyses of seizures obtained from CBD stores and to compare the results with the product declaration. Product declarations do not indicate the Cannabis variety, but state that the variety is listed in the EU PVC with the THC content less than 0.2%.

Cannabis samples were obtained from 46 seizures confiscated in the last year. Quantitative cannabinoids analysis was performed by GC-FID on Agilent 7890A GC System according to the accredited workflow. Extraction of DNA from plant material was performed using DNAeasy Plant Mini kit (Qiagen) following the manufacturer's protocol. DNA quantity was determined by Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). A portion of the tetrahydrocannabinolic acid synthase (THCAS) gene was amplified with primers "g" and "h" as outlined in (1) with the following modification. Briefly, protocol was optimized with duplex primer set using Qiagen Multiplex PCR kit. Furthermore, samples with detected THCAS marker were subjected to the D589 marker analysis with primers described in (2). PCR products were separated by capillary electrophoresis on AB 3500 GA. THCAS gene portion sequencing (265-1490bp) was conducted for samples with both markers detected along with two drug-type (positive controls) and two certified fiber-type varieties (negative controls). Sequencing was performed according to the laboratory standard operating procedures with primers described in (1) using Big Dye Terminator v.3.1 Cycle Sequencing Kit on AB3500xl GA (both from Life Technologies) and data analysis in Sequencher v.5.4.6. Sequences were aligned against the THCAS coding sequence of the drug-type cultivar Skunk (GenBank ID KJ469378).

Based on qualitative chemotype, 91.3% of samples were CBD (cannabidiol) predominant, and the remaining samples were CBG (cannabigerol) dominant. In 17 out of 46 tested samples, THC content was higher than 0.2 %. THCAS and D589 markers were detected in 12 analyzed samples. Eleven identical THCAS sequences were determined, which differed from the variants obtained in fiber-type except at the position 749. Moreover, G to A SNP at the 1064bp was observed. Moreover, one sample showed the sequence identical to the drug-type.

In 37% of total samples, THC content was above the value specified in product declaration. THCAS and D589 markers (specific for drug type varieties) were determined in 26.1% of tested samples. The complete concordance between abovementioned markers was observed, however their detection was not in agreement with the qualitative chemotype. Subsequent analysis demonstrated differences in THCAS sequences in comparison with the hemp sequences. Interestingly, 1064G→A substitution has been reported as a marker for THCA inactivation and CBGA dominance (3). However, this finding was not in correlation with the CBG content since it was found in only four tested samples. Hence, future studies will be focused on genetic analysis of complete THCAS and CBDAS genes in certified varieties and seizures.

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***Cannabis sativa L.*, THCA Synthase Gene, CBD Stores**

## B26 The Effects of Degradative Stress on the Headspace Profile of Fentanyl

Leann Forte, BS\*, Florida International University, Miami, FL; Stephanie Vaughan, PhD, Naval Research Laboratory, Washington, DC; Ashley Fulton, PhD, Naval Research Laboratory, Washington, DC; Lauryn DeGreeff, PhD, Florida International University, Miami, FL and Naval Research Laboratory, Washington, DC; Howard Holness, PhD, Florida International University, Miami, FL; Kenneth Furton, PhD, Florida International University, Miami, FL

**Learning Overview:** After attending, this presentation, attendees will understand how thermal, oxidative, and hygro-degradation (degradation due to moisture – i.e., humidity) affects the formation of volatile compounds in the headspace of fentanyl.

**Impact Statement:** This project will impact the forensic scientific community by providing data to aid in both the development of a non-contact detection method as well as training aid mimics in order to utilize canines as a detection tool.

Vapor detection, such as by canine or electronic nose, has the potential to serve as a vital non-contact detection method. Understanding a substance's unique vapor profile is imperative for development of any vapor detection method. Knowledge of any controlled substance's headspace profile can also be utilized to develop safe training aids for canine detectors making it easier to detect controlled substances in the field. This research focused on determination of the degradation products making up the headspace profile of fentanyl in order to better determine the active odorant for the development of a canine training aid mimic. After attending, this presentation, attendees will understand how thermal, oxidative, and hygro-degradation (degradation due to moisture – i.e., humidity) affects the formation of volatile compounds in the headspace of fentanyl. This project will impact the forensic scientific community by providing data to aid in both the development of a non-contact detection method as well as training aid mimics in order to utilize canines as a detection tool.

Fentanyl is a Schedule II synthetic opioid with a potency 100 times greater than that of morphine. It was first synthesized in 1960 by Dr. Paul Janssen and is used to treat severe, short-term, and chronic pain in cancer patients, or as an anesthetic.<sup>1</sup> The thermal and oxidative degradation of fentanyl was studied by Garg et al.<sup>2</sup> Using hydrogen peroxide for the oxidation studies, researchers identified the formation of fentanyl N-oxide. Thermal degradation at 350°C yielded N-phenylpropanamide (NPPA) and norfentanyl. In the work discussed herein, we discuss the effects of oxidative degradation due to ambient oxygen, thermal degradation at 40°C, and the presence of humidity on the evolution of components in the headspace of fentanyl. This investigation will inform both the development of a non-contact detection method as well as training aid mimics to utilize canines as a detection tool.

Solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) were used to determine the VOCs in the headspace profile of fentanyl. To determine which VOCs in fentanyl's headspace profile can be attributed to degradation, a series of experiments were performed using field-relevant environmental condition, examining degradation due to exposure to air, humidity, and heat. Fentanyl samples were left open under varying conditions to measure the effects of degradation due to humidity, temperature, and oxidative stress. Samples were left open for one week increments in the following conditions, 40 °C and ambient (22°C), inert or ambient air, and dry (3 %RH) or humidified (80 %RH).

Based on the results, NPPA was found to be the most notable product of degradation in all testing environments. There was also a notable increase in styrene after fentanyl was initially subjected to the 40°C environment for the first week, though this was followed by a decrease in styrene abundance in following weeks. Both styrene and NPPA were previously reported as a product of the thermal degradation of fentanyl.<sup>3</sup> Other compounds identified include, benzaldehyde, aniline, N-phenethyl-4-piperidone (NPP), heptane, benzeneacetylaldehyde, benzyl alcohol and 1-phenethyl-4-propinoloxy piperidine, which were not attributed to degradation. All decreased steadily over time in all four environments, except NPP which showed a slight increase following the 7 days of degradation and then remained constant. Understanding the headspace of fentanyl and the compounds that make it unique will be utilized to create safe, non-contact field detection methods.

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3. Nishikawa, Rona K.; Suzanne C. Bell; James C. Kraner; Patrick S. Callery. 2009. Potential biomarkers of smoked fentanyl utilizing pyrolysis gas chromatography-mass spectrometry. *Journal of Analytical Toxicology* 33: 418-22. <https://doi.org/10.1093/jat/33.8.418>.

### Fentanyl, VOCs, SPME-GC/MS

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## B27 Fentanyl Degradation in Syringes Obtained From Intravenous (IV) Drug Users in Washington, DC

*Shayla Montalvo, BA\*, George Mason University, Fairfax, VA; Alexandra Evans, MFS, DC Department of Forensic Sciences, Washington, DC; Morgan Levitas, MFS, DC Department of Forensic Sciences, Washington, DC; Luke Short, PhD, DC Department of Forensic Sciences, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will understand the degradation rate of fentanyl and common adulterants observed in used syringes in Washington, D.C. under natural degradation conditions following a data-drive approach. Additionally, attendees will be able to use the degradation rate to propose a time of use and improve testing methods for the detection of fentanyl in syringes collected from local needle-exchange programs and/or death investigations. Illicit fentanyl now drives the opioid epidemic and it is hypothesized herein that data from this research needs to be investigated to aid human health and can benefit analysis.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of fentanyl degradation within used syringes, with and without the adulteration of xylazine, etizolam and eutylone; common fentanyl adulterants detected in Washington, D.C. This research will offer a timeline of fentanyl stability for used syringes, adapting analysis times and/or storage protocols, and enhance treatment programs.

Needle-exchange programs provide drug users clean needles in exchange for used needles which helps lower exposure to blood-borne illnesses and other diseases. Analyzing the residual content captures current drug abuse trends within the intravenous (IV) user population. Information obtained from syringes associated with death investigations supports medicolegal inquiries by discovering what might have led to a fatal overdose. Information from syringes obtained from needle-exchange programs provides drug trend monitoring.

Most recent data illustrate the high prevalence of fentanyl in used syringes in Washington, D.C. Adulteration of fentanyl is not uncommon, with xylazine, etizolam and eutylone being adulterants of concern often observed. In our experience, syringes related to death investigations arrive at our laboratory for analysis days or even several weeks after the event occurred while syringes obtained from needle-exchange programs are delivered weekly, but are not time-stamped and definitive time of use ultimately remains unknown. The stability of compounds within a syringe can weaken over time depending on the syringe's storage conditions. Fentanyl in combination with cutting agents, may degrade at different rates and the presence of 4-ANPP can affect the analytical results of drug residue testing. If the analysis of syringes cannot be performed immediately, understanding the thermal byproducts and degradation rate of fentanyl combined with other substances can improve analytical methods for the detection of used syringes.

For this study, polypropylene syringes were conditioned to mimic used syringes among intravenous drug users in Washington, D.C. The controlled dangerous substances analyzed from syringes include the following, individually and in combination: fentanyl, 4-ANPP, etizolam, eutylone, and xylazine. Syringes were stored at ambient temperature (20-25°C) and degradation was observed over a 3-month time span, with every 7-day measurements of each analyzed drug starting at day zero, one, three days followed by every seven days. Methanolic extraction of drug residue was performed on each syringe, followed by gas chromatography mass spectrometry (GC-MS). Linear standard curves were run prior to each analysis with correlation values >0.99. Degradation was measured by quantifying the analytes and calculating the percent changed over time based off of data collected on day zero.

This presentation serves as a tool to estimate the timeline of degradation in fentanyl and its common adulterants seen in Washington, D.C. and identify any matrix interference or ion suppression that can affect detection of residues within syringes. This research will help Forensic and Public Health Laboratories improve current extraction procedures for the identification of CDS in syringes.

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### Degradation, Syringes, Fentanyl

## **B28 The Use of Novel Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Infrared Spectroscopy (GC/IR) Libraries for the Differentiation of Positional Isomers of Fentanyl-Related Substances (FRS)**

*Kimiko Ferguson, MSc\*, Florida International University, Miami, FL; Michael Gilbert, BS, Pinellas County Forensic Laboratory, Largo, FL; Reta Newman, MA, Pinellas County Forensic Laboratory, Largo, FL; Jose Almirall, PhD, Florida International University, Miami, FL*

**Learning Overview:** After attending this poster presentation, attendees will be able to better understand the difficulties of fentanyl analysis using electron impact GCMS and the benefits of using GC-IR in the identification and differentiation of fentanyl related substances (FRS).

**Impact Statement:** The forensic community will be presented with an alternative for the unambiguous identification of fentanyl related substances using GC-IR and the results of a validation study and database search exercise for fentanyl related substances using novel GC-MS and GC-IR libraries.

Forensic laboratories routinely use gas chromatography mass spectrometry (GC-MS) in the identification of controlled substances but certain drug classes, such as the positional isomers of fentanyl related substances, produce indistinguishable electron impact ionization mass spectra. The core structure of fentanyl, now the fourth most identified drug in the United States<sup>1</sup>, consists of an amide group, a piperidine ring, an aniline ring, and an N-alkyl chain, each providing opportunities for points of substitution that create FRS and corresponding positional isomers.<sup>1,2</sup> For this study, the analysis by GC coupled to a vapor-phase infrared spectroscopy detector (GC-IRD) was used as a complementary technique to GC-MS for the identification of positional isomers of FRS.<sup>3</sup> The result is a novel fentanyl library consisting of more than 210 reference compounds. A collaboration among three different laboratories yielded 100% correct identifications of each of the 212 analogs when the new GC-IRD fentanyl library created at Florida International University was used. This compares to 15.1% correct identification when using another, more limited, GC-IRD library and 4.7% correct identification when using an existing and widely used GC-MS library alone. The results from interlaboratory studies among six different laboratories demonstrate the utility of the libraries in assisting forensic laboratories in the identification of fentanyl-related compounds. This newly created GC-IRD library and its GC-MS counterpart of 212 fentanyl-related substances will be made freely available to the forensic science community.

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### **Positional Isomers, Fentanyl-Related Substances, Spectroscopy**

## **B29 The Application of Electrospun Polymers with Metallic Nanoparticles for Use in the Detection of Fentanyl by Surface-Enhanced Raman Spectroscopy (SERS)**

Daniel Rubin, BA\*, Florida International University, Miami, FL; Mario Vendrell-Dones, MS, Florida International University, Miami, FL; Ling Wang, PhD, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL

**Learning Overview:** After attending the presentation, attendees will see how combining electrospun nanofibers with nanoparticles can be used to enhance the surface-enhanced Raman spectra for fentanyls.

**Impact Statement:** This method will allow the forensic analyst to work quickly and efficiently, in order to identify the substances that pose a greater health risk to society.

The current opioid epidemic began in the 1990s with doctors prescribing opioids to help patients manage pain after surgical procedures.<sup>1</sup> From 1999 to 2019 it is estimated that around 500,000 people have died from overdosing on different opioids including prescription opioids and illicit synthetic opioids.<sup>2</sup> Fentanyl is a schedule II drug that is 50-100x more potent than morphine. Due its potency, it can only be prescribed by doctors as a painkiller in very specific situations. However, fentanyl is commonly subverted for recreational use. In 2019, it was estimated that more than 36,000 users died by synthetic opioids alone, which is 12 times the estimated amount from 2013.<sup>3</sup>

The rise in drug overdose has led the research community to produce new, and quicker detection methods. Sensors which can quickly detect structurally similar drugs and its concentration can be very instrumental in emergency room settings. One method suggested to do this has been to use Raman Spectroscopy to determine unknown drugs of abuse. Raman Spectroscopy works by detecting the interaction between inelastically scattered light and the polarizability of molecular vibrational bonds. The scattered light interacts with these molecular bonds resulting in a unique spectrum for each compound. The inelastically scattered light may be anti-Stokes or Stokes scattering in energy resulting in two separate but equivalent spectra.<sup>4</sup> The problem with this method is that only a small proportion of the scattered light is inelastically scattered. Thus, Raman spectroscopy is not sensitive enough to detect drugs at the needed low concentrations (< ng/mL). To combat this relatively low sensitivity, gold, or silver metallic nanoparticles can be added to the surface to enhance the signal from the analyte. The metallic nanoparticles help enhance the signal through an interaction between the localized surface plasmon field of the metal, the adsorbed molecules, and the scattered light. This enhancement is referred to as Surface-enhanced Raman Spectroscopy (SERS).

Nanoparticles can be synthesized in a variety of methods. The method used in this project is the seed mediated method as it gives the most control over the final shape of the nanoparticle.<sup>5</sup> The ideal shape for SERS is star shaped as it gives the greatest Raman enhancement.<sup>6</sup> In order to make the nanostars Deionized water was reacted with H<sub>2</sub>AuCl<sub>4</sub> 10<sup>-2</sup> M, AgNO<sub>3</sub> 10<sup>-2</sup> M, L-AA 10<sup>-1</sup> M, and Na<sub>2</sub>CO<sub>3</sub> 1M. Recently, it has been proposed that nanofibers can be bound to metallic nanoparticles to be used as a surface to enhance the signal of organic compounds.<sup>7</sup> In order to incorporate the nanostars, they are mixed into the nanofiber fiber solution at a 1:1 ratio. The nanofiber solution is polyvinylpyrrolidone dissolved in ethanol at 9-10% by weight. Once the solution has been made it will be electrospun. In this process a high voltage is used to draw a charged polymer through a syringe. The process gets its name from the fact that an applied voltage creates a magnetic field so when the polymer is pulled through a fine needle the viscous liquid spins creating nanofibers.

The present research project developed a method to incorporate nanoparticles into the electrospinning procedure to make nanofibers that can be used in SERS. This process will allow forensic analysts to detect benzylfentanyl, and fentanyl at the ng/mL concentration level. This method will allow the forensic analyst to work quickly and efficiently, in order to identify the substances that pose a greater health risk to society.

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### **Electrospinning, Nanoparticles, SERS**



## **B30 Identifying the Similarity Between In-Source Collision-Induced Dissociation (IS-CID) Fragment Ion Spectra and Tandem Mass Spectrometry (MS/MS) Product Ion Spectra for Seized Drug Identifications**

*Jared Sharp, BS\*, Sam Houston University, Huntsville, TX; Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX*

**Learning Overview:** After attending this presentation, attendees will have learned about the mass spectral similarity between IS-CID fragment ion spectra and MS/MS product ion spectra of representative synthetic cathinones, synthetic cannabinoids, and fentanyl analogs. The audience will learn about the application of IS-CID for the generation of fragment ion spectra, and methods to assess mass spectral similarity including breakdown curves, Pearson product-moment correlations (PPMCs), and simple match factors (SMFs).

**Impact Statement:** This presentation will impact the forensic science community by providing a deeper understanding of the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra and provide additional scientific basis for the use of IS-CID within the forensic science community.

The application of IS-CID for the generation of fragment ion spectra has become increasingly popular with soft ionization techniques, such as direct analysis in real time (DART<sup>®</sup>), when coupled to single-stage high-resolution mass spectrometers. The mass spectra generated by soft ionization techniques contain predominately protonated or deprotonated precursor molecules, which must be fragmented to gain additional selectivity and structural information. However, there is currently only limited information available about the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra, which limits the reliability of IS-CID for the identification of seized drugs. The central hypothesis is that under optimized conditions, IS-CID fragment ion spectra can be generated that are highly similar to MS/MS product ion spectra, which is essential for the further development of IS-CID applications to seized drug identifications.

An Agilent 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer was operated with direct infusion electrospray ionization (ESI) for the analysis of representative synthetic cathinones, synthetic cannabinoids, and fentanyl analogs. The combination of direct infusion with ESI and a Q-TOF mass spectrometer provided the capability to rapidly switch between different source voltages and collision energies to collect data for IS-CID and MS/MS under conditions as similar as possible. The IS-CID fragmentation was collected over a fragmentor voltage range of 150 V to 350 V in increments of 10 V with a constant skimmer voltage of 65 V. In comparison, the MS/MS activation was collected with collision energies ranging from 0 eV to 40 eV in 2 eV increments. The compounds analyzed in this study include: 4-chloroethcathinone (4-CEC), pentylone, 3-methoxymethcathinone (3-MeOMC),  $\alpha$ -PVP, AB-PINACA, AB-FUBINACA, MAB-CHMINACA, AM-2201, fentanyl, carfentanil,  $\alpha$ -methylfentanyl, and alfentanil.

The breakdown curves, PPMC, and SMF were performed through Microsoft Excel. Breakdown curves provide a visual assessment of the relationship between the applied activation energy and the resulting mass spectra through a comparison of the major fragment ions and their corresponding abundances. In comparison, PPMC and SMF are similarity metrics that provide a numerical assessment of the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra under given conditions.

The results from the breakdown curve analysis generated from IS-CID and MS/MS data indicate a similar slope and overall shape between the majority of analyzed compounds, which highlights the similar fragmentation behavior observed between these two activation techniques. Likewise, the PPMC and SMF results for each possible comparison of IS-CID and MS/MS activation conditions indicate that there are conditions under which highly similar IS-CID fragment ion spectra and MS/MS product ion spectra are generated. For example, the average highest PPMC coefficient for each comparison between IS-CID and MS/MS activation conditions ranged between 0.9314 and 0.9567, indicating a high degree of spectral similarity. Additionally, the average SMF result for the highest similarity conditions between IS-CID and MS/MS activation conditions ranged from 687-829, indicating a good match between the resulting mass spectra.

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### **Seized Drugs, Mass Spectral Similarity, Fragmentation Behavior**

## B31 Silver-Doped Nanofibers as Surface-Enhanced Raman Spectroscopy (SERS) Substrates for the Detection of Synthetic Cathinones

Mario Vendrell-Dones, MS\*, Florida International University, Miami, FL; Daniel Rubin, BA, Florida International University, Miami, FL; Ling Wang, PhD, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL

**Learning Overview:** After the presentation, attendees will understand the principles of Surface-Enhanced Raman Spectroscopy (SERS), how nanomaterials/nanofibers can be used to increase the sensitivity of the obtained signal and how it can be applied to rapidly detect synthetic cathinones.

**Impact Statement:** This work is intended to impact the forensic science community by the detection of substances that are a risk to society in a faster and more reliable way.

This work is intended to impact the forensic science community by the detection of substances that are a risk to society in a faster and more reliable way. In the distant past, indigenous people around the horn of Africa began chewing leaves of the khat plant for relief from daily toil. This habit has now resulted in the discovery of numerous compounds of medical use known as cathinones. Unfortunately, it has also resulted in the development of countless structural variations of addictive analogues. Research into the effects that these compounds have on humans is lacking due to the rapidity at which new compounds become available in the market, resulting in problems for policing and health agencies. Moreover, manufacturers synthesize these new synthetic cathinones in numerous and often amateur ways, causing variations in their purity and the presence of reaction by-products and additives, ultimately increasing the risk of overdose for users.<sup>1</sup> Therefore, there is a pressing need for a screening method that can assist authorities in recognizing various cathinones in a rapid, sensitive, and inexpensive way. Due to its molecular specificity and sensitivity, Surface enhanced Raman Spectroscopy (SERS), constitutes an ideal and flexible platform to detect newly developed compounds and differentiate analogs. This work describes the fabrication and optimization of nanofibers for use as enhancing substrates for the SERS detection of two model cathinones, 3,4-methylenedioxy-5-methylethcathinone and 4-methylethcathinone.<sup>2-4</sup> The SERS substrate is developed by combining colloidal silver (Ag)-based nanoparticles with water soluble polymers, followed by electrospinning the solution into composite nanofibers, and finally crosslinking the nanofibers to reduce solubility and increase the density of hot spots on the surface.<sup>5</sup> Since the intensity of SERS signal depends on the proximity of the analyte to the nanometallic surface and its vibrational interaction with the electromagnetic field generated by the oscillating electrons of the metal (i.e., LSPR), the quality of the analyte adsorption will affect the overall outcome of the experiment. pH experiments were performed to determine optimal charge states for both surface and analyte. Our results indicated that higher signal enhancements and lower detection limits were achieved at elevated pH. Additional experiments involving Density Functional Theory (DFT) calculations at the B3LYP/6-311G\*\* level were utilized to predict the Raman frequencies of the studied compounds and assist in the assignment of their vibrational modes.<sup>6</sup> In parallel, traditional Raman measurements on dried solid residues of the synthetic cathinones standards were examined and the resulting spectra were used as references to ensure concordance of the DFT-predicted frequencies with experimental values. Within this work, a rapid, sensitive, and alternative trace detection method of novel psychoactive substances, such as synthetic cathinones, is presented by combining the use of nanofibers and colloidal solutions.

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### Synthetic Cathinones, SERS, Nanofibers

## B32 The Extraction of Cannabidiol (CBD) From Gelatin Edibles With Analysis by Paper Spray Ionization-Mass Spectrometry (PSI-MS)

*Cheyenne Granger, BA\*, Duquesne University, Pittsburgh, PA*

**Learning Overview:** After viewing this presentation, the audience will have a better understanding of how the rise of cannabis products may impact Forensic Science, how we can significantly improve CBD edible analysis, and how paper-spray ionization mass spectrometry may be the future of rapid edible drug detection.

**Impact Statement:** This research project will impact the forensic science community by presenting a new and likely more efficient technique (SIPSI-MS) for CBD gelatin edible analysis in crime laboratories.

In the past two decades, the United States has observed a rise in the use and consumption of cannabis related products.<sup>1</sup> This rise comes as a result of increased popularity and 36 of the 50 states legalizing the use of these products.<sup>2</sup> With this observed increase, an influx of cannabis related evidence will likely be seen in crime laboratories across the country. This influx of evidence would likely cause a significant disruption to evidence intake and analysis leading to a greater chance of backlogging. For gelatin CBD (cannabidiol) edibles, a popular cannabis food product, the current methods of analysis may quickly become inhibitory due to their lengthy preparation and analysis times. The current methods include HPLC (high performance liquid chromatography) and GC-MS (gas chromatography mass spectrometry), the latter of which requires derivatization.<sup>3</sup> While these methods are extremely useful, the use of PSI-MS (paper spray ionization mass spectrometry) may provide increased efficiency of identification and quantification of the CBD content in gelatin edibles. For this research project, a technique called SIPSI-MS (silver impregnated PSI-MS) was developed for the rapid detection and possible quantification of CBD content in CBD gelatin edibles. Through this technique, a more efficient identification of CBD extracted from a gelatin matrix was observed. The quantification of the extracted CBD from a gelatin matrix will likely have similar efficiency to identification as indicated by preliminary testing. Overall, this research may demonstrate that there could be significant improvement to CBD gelatin edible analysis via SIPSIMS, if the equipment is available at crime laboratories. This will allow for crime labs to lower the cost and time needed for CBD analysis from gelatin matrices, and in addition may provide a significant solution to offset any backlogging created by increased cannabis usage. This research may also be considered significant for the quality assurance and purity issues observed in cannabis edibles.<sup>4</sup> Further research with SIPSI-MS for identification and quantification of THC (tetrahydrocannabinol) from edibles may further contribute to forensic analysis of cannabis products.

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### Cannabidiol, Edibles, Mass Spectrometry

## **B33 The Quantitation of Cannabinoids in *Cannabis Sativa L.* Plants from Pakistan Using Gas Chromatography/Flame Ionization Detector (GC/FID)**

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**Learning Overview:** The attendee will learn about the quantitation of cannabinoids in Cannabis and method development & validation using GC-FID technique specifically for forensic analysis. The study will also help in determining the Cannabinoid concentration in cannabis situated at various areas of Pakistan thus helping phenotyping and age determination.

**Impact Statement:** This study will provide forensic community an overview of the cannabis constituents present in Cannabis plant of Pakistan origin. It will also focus on, method validation, extraction & quantitation procedure for forensic analysis.

Cannabis (*Cannabis sativa L.*) and cannabis products are commonly abused in North Africa, South-West Asia, particularly Afghanistan and Pakistan. It belongs to Cannabinaceae family. It is given various street names/synonyms like Garda, Charas, Ganja, hemp, marihuana, marijuana, pot, gandia, grass etc. Various psychoactive compounds called cannabinoids are produced in trichomes. The most common cannabinoids include Cannabidiol (CBD), Tetrahydrocannabinol (THC & D<sup>9</sup>-THC) and Cannabinol (CBN). D<sup>9</sup>-THC occur as 10-12 % in pistillate flowers, 1-2 % in leaves, 0.1-0.3 % in stalks and < 0.03 % in the roots. Prolonged use of cannabis may affect cardiovascular, respiratory and focal sensory systems.

The objective of conducting this study was to develop and validate method for quantitation of Cannabinoids (CBD, THC and CBN) that can be used in forensic analysis. The plants were collected from different regions of Pakistan and their concentration were calculated using internal standard (IS) method.

**Materials and Methods:** Samples of cannabis plants were collected from different regions of Pakistan like Azad Kashmir, Punjab KPK, Islamabad etc. Shade dried leaves & fruiting tops were used for extraction & analysis on GC-FID for cannabinoids determination. Validation studies were carried out using a known range (10, 20, 40, 60, 80 µg/mL) of each of ethanolic certified standards and unknown samples were extrapolated alongside using Phenanthroline as IS. An Agilent GC coupled with Flame ionization detector (7890B GC, ALS G4513A, Agilent Technologies, USA) was used for this purpose. Quality controls 25 µg/ml and 65 µg/ml & negative control spiked with 110 µl (55 µg/mL) IS solution were dried reconstituted with 200 µl of methanol were used. For validation, Linearity, Accuracy, Precision, Carry over, Interference, LOD and LOQ were evaluated. A mixture of different available drug standards containing GHB, Amphetamine, Starch, Phenaramine, PCP, and methadone, Fentanyl, Dextromethorphone and Methamphetamine with IS were analyzed to determine the absence of common interferences from the matrix. Accuracy and precision were determined by using 30 µg/ml of each standard containing IS & run replicates for five days.

**Extraction:** Dried leaves (100 mg) and fruiting tops (50 mg) were extracted with 2200 µl of 500 ppm of Phenanthroline (IS) & ethanolic (as solvent) extracts were dried on turbopap evaporator using N<sub>2</sub> gas at 40°C. Reconstituted the tubes with 200 µl of methanol and vortex.

**GC-FID Parameters:** Sample were injected (2 µL) in Agilent 30m x 250 µm x 0.25 µm DB.5 MS column in split mode (50:1). Oven temperature was programmed at 150 °C up to 300 °C with variable ramping & total run time of 10.5 minutes. The FID front detector with Heater at 250 °C & Nitrogen (99.999% pure) as carrier gas was used. The H<sub>2</sub> gas was used as igniting gas. The data was evaluated by Chem Station calculated regression coefficient with auto integration parameters.

**Results and Conclusion:** The cannabinoid concentration was calculated in leaves and fruiting tops of cannabis. The retention time (RT) for CBD, THC, CBN and Phenanthroline was determined as 7.942 ±0.2, 8.385±0.2, 8.701±0.2, and 6.628±0.2 minutes, respectively. Linearity was calculated with regression coefficient as 0.9992 (CBD), 0.9998 for THC and 0.9997 for CBN. The method showed no interference & LOD (10 µg/ml). The method was accurate and precise with <20 % acceptance criteria. The quantity of cannabinoids was expressed as the mean values of dry weight expressed as µg/mL.

**Conclusion:** The Cannabinoids content in cannabis plant of Pakistan showed marked variation. The validation and quantitation of the principal cannabinoids have great importance in pharmacological and forensic studies. The method is simple & reliable & has various applications, Future prospect of this study is to establish correlation between cannabinoid concentrations and age of plant.

### **Forensic Analysis, Cannabis, GC/FID**

## B34 The Quantitation of Opium Alkaloids in Seized, Crushed, and Mowed Plant Material

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**Learning Overview:** After attending this presentation, attendees will be able to learn: (1) how the contents of crushed *Papaver somniferum* L. can be extracted and confirmed using GC/MS after derivitization; (2) about challenges faced in confirmation using SIM mode; (3) use of alternative procedure of extraction instead of expensive solid phase extraction procedure.

**Impact Statement:** Forensic science community will be able to use this validated method for confirmation of opium alkaloids when conventional GC/MS or GC/FID give non reproducible results. This method will be particularly useful when concentration of alkaloids is low in mowed plant material from which juice has been extracted.

*Papaver somniferum* L. is herbaceous flowering plant. All or parts of the plants (except seeds) in any form are controlled as per law in Pakistan. Pakistan is poppy free country but being immediate neighbour to Afghanistan, there is continuous trafficking of poppy plant and opium alkaloids in Pakistan. Furthermore, use of crushed plant material is famous for its medicinal value as well. In Pakistan, opium and poppy plant seizure comprise of nearly 06% of total seizures per year. Author's laboratory routinely face problems in confirmation of opium alkaloids from crushed dried poppy plant materials from which alkaloids have already been extracted. Therefore, there was a need to develop and validate the robust method by which opium alkaloids can be confirmed and quantitated using GC/MS.

Agilent GC-7890A with MS-5975C (20m x 180um x 0.18um DB-5 MS UI column) was used in this study. Methanol (1 mL) was added in tube containing finely crushed poppy plant material (20 mg) along with 30 uL of IS solution (Phenanthroline 1mg.mL<sup>-1</sup>). Calibrator solutions (1 ml in methanol) of codeine, morphine, thebaine and papaverine (5 to 50 ppm), positive control (25 ppm) and negative control were spiked with 30 uL of IS solution. Samples were sonicated for 30 minutes, centrifuged and methanol was evaporated after separation. Residue was derivitized with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA):ethyl acetate (25:75 uL) and heated in capped conical glass tube for 30 minutes at 70-80°C. Derivitized solutions were cool down to room temperature and 1 uL was injected to 10:1 split GC-inlet (250°C) using SIM mode (one quantifier and two qualifier ions of each analyte) and helium as carrier gas. Oven temperature was programmed initially at 150°C for 1 min, ramped at 25°C/min up to 250 °C and finally ramped at 10°C/min to 300°C (Total run time 10 min). Method was validated for linearity, selectivity, LOD, LOQ, bias, precision and matrix effect as per UNODC guidelines.

IS, codeine, morphine, thebaine and papaverine were detected with sufficient resolution having retention times of 4.9, 6.4, 6.6, 6.8 and 7.9 min, respectively. Separation of analyte peaks is dependent on oven temperature ramping. Response of all analytes is linear over calibration range (regression coefficient >0.995). Lowest calibrated was set as LOQ of method while LOD ranges from 31.25 to 125 ug/g. No interference is observed with common adulterants and controlled substances. Precision and bias are well below 20% for all analytes. Twenty four case samples were also analysed and produced reproducible results.

Based on the study, it is concluded that this method is sufficiently specific, selective and reproducible with enough LOQ for confirmation and quantification of opium alkaloids in challenging case samples.

### Poppy, Opium, Confirmation

## B35 The Use of Urine Rapid Response Fentanyl Test Strips to Detect Fentanyl in Seized Substances

Lauren Bishop\*, Ohio Northern University, Ada, OH

**Learning Overview:** The learning overview in this presentation demonstrate that these lateral flow immunoassay strips work in non-laboratory settings for detection of fentanyl and fentanyl analogues mixed in seized substances. As well as a method for harm reduction to be distributed by social service agencies along with the test strips so it's easy to follow for people who use drugs.

**Impact Statement:** This study validates the use of urine Fentanyl test strips for detection of fentanyl and fentanyl analogues. This will impact the forensic science community by practically testing these strips and allowing them to be put in a real life setting to examine if the strips really work for a new intended use.

According to the Center for Disease Control (CDC), since 1999, nearly 841,000 people have died from a drug overdose.<sup>1</sup> In 2021 the Montgomery County Coroner's office in Ohio has recorded 235 overdose deaths until August 30<sup>th</sup>, 2021, and 323 overdose deaths in 2020, with 88% of those related to fentanyl.<sup>2</sup> Fentanyl is being mixed with other illicit substances according to the DEA.<sup>3</sup> This study was designed to determine if Rapid Response™ urine fentanyl test strips would accurately detect fentanyl in seized substances and was set up to imitate the setting of people who use drugs, as opposed to a laboratory setting. Most of the published research surrounding the test strip efficacy has focused on testing the strips on pure standards or singular substances, which leaves uncertainty regarding true street substance samples that have been adulterated and whether mixtures routinely seen in casework would ultimately affect the results. Harm reduction agencies such as public health and coroner's offices in Montgomery County and Cleveland, Ohio, as well as in other states like Washington, along with organizations like Dance Safe, are proponents of user testing.<sup>4,5</sup> All these agencies have attempted to implement different programs to help reduce fentanyl overdose deaths. The reason for the focus around fentanyl is because fentanyl about 100x more potent than morphine. The intent of the test strips is to detect fentanyl mixed in with a user's illicit substances before taking them. This then gives the user the knowledge and the ability to decide whether to take or not take the illicit substance. Harm reduction agencies are distributing the Rapid Response™ fentanyl test strip to people to test their various substances to determine if they contain fentanyl to avoid the potential for overdose. The samples tested in this study were from 76 cases submitted to the Miami Valley Regional Crime Lab in Dayton, Ohio. The detection of fentanyl and other illicit substances was qualitatively determined on a Gas Chromatography-Mass Spectrometry (GC/MS) to confirm the presence and abundance of each substance prior to strip testing. The manufacturer and supplier of the strips is BTNX Inc. located in Markham, Ontario, Canada. These test strips are based on the principle of lateral-flow immunoassay (LFIA), as a competitive assay between drug conjugates and free drug present. So, in this assay, the results are inversely related to the signal and have a sensitivity or a cutoff concentration of 20ng/mL.<sup>6</sup> The variables investigated in the study included: the water type, (Dayton, Ohio city tap water), the seized drug substances (cases from Ohio and Indiana), mass of powder substance used (less than 0.01g) and the length of time the strips were exposed to the mixture and lastly the length of time lapsed prior to reading results (per the manufacturer instruction). The results from 73 of the 76 cases indicated positive detection demonstrating the presence of fentanyl, regardless of the abundance of each controlled substance versus adulterants. However, there were three cases that did not contain fentanyl as determined by GC/MS, but did contain heroin and/or methamphetamine, which gave unexpected results. Two of the cases were false positives, and one case was negative. These three cases were tested multiple times and it was concluded the results were accepted. This study demonstrates that the Rapid Response™ fentanyl test strips are sensitive about 96% of the time and as tested would be a reasonable method in the hands of people who use drugs to limit the exposure to fentanyl when using illicit drugs.

### Reference(s):

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### Fentanyl, Lateral-Flow Immunoassay, Opioids

### B36 Continuous Vapor Sampling of Volatile Organic Compounds (VOCs) Associated With Explosives and Cocaine Using Capillary Microextraction of Volatiles (CMV)

*Jacqueline Rodriguez, MS\*, Florida International University, Miami, FL; Jose Almirall, PhD, Florida International University, Miami, FL*

**Learning Overview:** After attending this presentation, attendees will learn about a novel continuous vapor delivery and sampling system which can be implemented to rapidly sample the vapors of illicit drug and explosive analytes by using the CMV.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how the vapors of sub-nanogram amounts of VOCs, which are associated with explosives and cocaine, were capable of being rapidly recovered and detected by coupling the CMV device to portable instrumentations, indicating the potential for field applications.

The dynamic sampling and preconcentration device, capillary microextraction of volatiles (CMV), is coupled to portable instrumentation for the rapid field detection of volatile organic compounds (VOCs) associated with drugs and explosives. By coupling the CMV device to a portable gas chromatograph-mass spectrometer (GC-MS), sub-nanogram (ng) instrumental detection limits were achieved for VOCs associated with cocaine and VOCs associated with smokeless powder explosives, including 3-nitrotoluene (3-NT), 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), ethyl centralite (EC), dibutyl phthalate (DBP), and 2-nitrodiphenylamine (2-NDPA). Three different dynamic sampling methods were used with the CMV to sample and preconcentrate the volatiles prior to analysis. The headspace of a closed system was sampled over 10 minutes resulting in recoveries for the explosives analytes between 0.3 – 12% by subsequent GC-MS analysis using a field-portable Griffin G510 GC-MS. Simulated open-air vapor sampling was conducted using a previously described vapor source, which resulted in an improvement of analyte recovery (ranging from 1.6 – 25%), after sampling for 10 minutes. A novel, continuous vapor delivery and sampling system was used, for the first time, to facilitate the gradual delivery of sub-nanogram quantities of the VOC analytes of interest. The new continuous delivery system achieved significantly higher recoveries (between 3.0 – 89%) for all the analytes, while requiring less sampling time (~ 5 minutes) and sampling volumes. Each of the sampling methods presented attained an overall analytical precision of better than 14% relative standard deviation (RSD) for mass loadings as low as 100 ng/l. The VOCs that were studied for drug analysis derive from analytes that are commonly associated with the presence of cocaine, including methyl benzoate, methyl cinnamate, anhydroecgonine methyl ester (AEME), ecgonine methyl ester (EME), and benzocaine. Throughout this study, the rapid sampling and preconcentration of these VOCs followed by a ~ 10-minute portable GC-MS method, compared favorably to the analytical figures of merit achieved by laboratory instruments and the detection limits reported for canines. Another portable instrumentation, a hand-held ion mobility spectrometer (IMS), was also utilized for the application of field vapor detection of explosives and illicit drugs. The portable IMS possesses a rapid analysis time of only ~ 20 ms and was coupled to a planar solid phase microextraction (PSPME) disk to accommodate for the configuration of the IMS inlet. For vapor sampling, the circular PSPME disk was placed on top of the inlet to allow the analytes to adsorb onto the coated (phenyl-terminated polydimethylsiloxane) microfibers within the sampling device prior to analysis of the IMS. These new, fast, and sensitive sampling methodologies coupled to the rapid analysis of field-portable instruments expands the potential applications of the CMV for the field detection of trace analytes associated with illicit drugs and explosives. References: 1. JL Rodriguez and JR Almirall, Continuous vapor sampling of volatile organic compounds associated with explosives using Capillary Microextraction of Volatiles (CMV) coupled to a portable GC-MS, 2021, For. Chem., submitted.

#### Capillary Microextraction of Volatiles (CMV), Volatiles Organic Compounds (VOCs), Portable Instrumentations

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## **B37 An Assessment of Robotic Solid Phase and Immunoaffinity Microextraction With Intact Mass Analysis for Saliva Identification in Forensic Samples**

*Catherine Brown, PhD, University of Denver, Denver, CO; Phillip Danielson, PhD, University of Denver, Denver, CO; Kevin Legg, PhD\*, Center for Forensic Science Research & Education, Willow Grove, PA*

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of the advances in protein analysis for the characterization of human saliva by intact mass spectrometry.

**Impact Statement:** This presentation will benefit the forensic science community by providing a rigorous scientific evaluation of two automated extraction techniques for the recovery of human statherin (a saliva biomarker) from forensic samples.

Alpha amylase is the most common target for salivary identification. While abundant in saliva, amylase is also detectable in vaginal secretions, sweat, and breast milk. Traditional enzyme-activity and immunochromatographic assays provide only a presumptive indication of saliva. In an effort to overcome these specificity limitations, several novel strategies have been explored including assays based on mRNA, epigenetics, Raman spectroscopy, and protein mass spectrometry.

Several previously established mass spectrometry-based approaches have been developed. While reliable, most are limited in throughput due to multi-step workflows that include protein extraction, quantitation, enzymatic digestion, solid phase cleanup, and nano/capillary-based chromatography. The work presented here has developed and assessed two “direct”, single stage, automated extraction strategies including immunoaffinity purification and reverse phase solid phase microextraction in conjunction with intact mass analysis of human statherin.

Swabs or cuttings were reconstituted in 500  $\mu$ L of PBS buffer at room temperature and centrifuged. Supernatant (125  $\mu$ L) was diluted 1:1 in 1 mg/mL BSA in PBS for immunopurification or 0.5% TFA in water for C18 solid phase extraction. All extractions were performed on an Agilent Technologies AssayMAP Bravo liquid handling platform with 5  $\mu$ L C18 or streptavidin cartridges. Biotinylated ProteinTech STATH polyclonal mouse anti-Statherin antibody was employed for immunoaffinity purification. Mass analysis was performed on the Thermo Q-Exactive mass spectrometer operated in positive Full-MS mode. Separation was carried out at 0.3 mL/min over an 8-minute gradient separation. Data analysis was performed with BioPharma Finder v4.1 using the Xtract algorithm to deconvolute intact statherin.

Two sample sets were analyzed including a population of 20 individuals to evaluate detection consistency as well as a series of casework samples to evaluate assay performance. Of 20 individuals sampled, statherin was identified in 18 samples by immunoaffinity purification and 17 samples by C18 extraction. A total of 26 casework type samples were assayed including mixed and single-source samples differing in age, substrate type, and type of chemical and environmental insult. All casework type samples were analyzed in duplicate for a total of 104 samples. Statherin was confidently identified in 94% of samples extracted using the immunopurification approach and 65% of samples by the C18 extraction. Both methods detected sub-microliter quantities of saliva applied to cotton swabs. Samples treated with a 10% bleach solution failed to be detected using either extraction method. This was the only sample type to fail with the immunopurification approach. All sample mixtures including saliva mixed with blood or semen as well as saliva fortified on menstrual swabs were not detectable using the C18 method.

Overall, immunoaffinity purification outperformed C18 extraction; especially when analyzing complex mixtures. This is likely due to the ability of immunoaffinity purification to generate highly purified extracts which circumvent the suppressive effects of non-target proteins. Conversely, while immunoaffinity purification performed well, these assays require a significant amount of development to identify appropriate antibodies, incubation periods, binding and wash conditions compared to SPE-based methods. In conclusion, robotic extraction and intact mass spectrometry can provide serological identification of human saliva from a variety of real-world sample types.

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### **Serology, Intact Protein Analysis, Mass Spectrometry**



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**B38 The Separation of Natural and Synthetic Hallucinogenic Tryptamines Using High-Performance Liquid Chromatography-Photodiode Array (HPLC-PDA) and Ultra-High Performance Liquid Chromatography-Photodiode Array/Mass Spectrometry (UHPLC-PDA/QDa)**

*Mia Fabbri, BS\*, Cedar Crest College, Allentown, PA; Marianne Staretz, PhD, Cedar Crest College, Allentown, PA; Matthew Wood, PhD, Ocean County Sheriff's Office, Toms River, NJ; Daniel Garrison, BS, Waters Corporation, Plymouth Meeting, PA; Peter Harrsch, PhD, Waters Corporation, Plymouth Meeting, PA; Thomas Brettell, PhD, Cedar Crest College, Allentown, PA*

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**Learning Overview:** The goal of this presentation is to report the results of the development of a method to separate 12 natural and synthetic hallucinogenic tryptamines using high performance liquid chromatography-photodiode array (HPLC-PDA) and ultra-high performance liquid chromatography-photodiode array/mass spectrometry (UHPLC-PDA/QDa).

**Impact Statement:** This presentation will impact the forensic community by demonstrating that UHPLC-PDA/QDa can be a powerful and viable alternative tool in the analysis of natural and synthetic hallucinogenic tryptamines and that HPLC-PDA can also be used to separate 12 hallucinogenic tryptamines.

Every year new psychoactive substances (NPS) emerge on the illicit market and most end up being submitted to crime laboratories as seized drugs for analysis. Tryptamines are one of these common classes of NPS. Synthetic tryptamines are derived from natural tryptamines such as psilocybin and N,N-dimethyltryptamine (DMT). Since these natural tryptamines are altered to form new compounds, the NPSs are structurally similar and can therefore be difficult to distinguish and identify. The most used method for seized drug analysis is gas chromatography-mass spectrometry (GC-MS), however, this method requires that the compounds be thermally volatilized. This is not ideal for tryptamines since psilocybin can be dephosphorylated to psilocin in high temperature, acidic, or basic environments. Therefore, a more accurate method is necessary to detect the parent compound for tryptamine identification. HPLC-UV offers a non-degradative solution with adequate sensitivity for tryptamine analysis. The addition of mass detection enhances the capability of being able to identify seized drugs and should be very useful in the detection of tryptamines.

Twelve psychoactive tryptamine standards were prepared in methanol and studied using both HPLC-PDA and UHPLC-PDA/QDa. These included the three most common tryptamines: DMT, 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT), and N,N-diallyl-5-methoxytryptamine (5-MeO-DALT); and four natural psychoactive tryptamines: psilocybin, psilocin, bufotenine, and ibogaine. Additionally, 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT), 5-methoxy-alpha-methyltryptamine (5-MeO-AMT), 5-methoxy-N-methyl-Nisopropyltryptamine (5-MeO-MiPT), N,N-dipropyltryptamine (DPT), and N,N-diethyltryptamine (DET) were analyzed. Retention time data, as well as UV spectra and selected ion recording (SIR) data were obtained for each individual compound using HPLC-PDA and UPLC/PDA-QDa. Both methods used binary mobile phases with gradients. The HPLC-PDA method mobile phase conditions consisted of 90% A (0.1% TFA in water) and 10% B (0.1% TFA in 2:1 acetonitrile to methanol) with a gradient elution up to 40% B at 0.4 mL/min using a Raptor® Biphenyl 5- $\mu$ m column (100 mm x 3 mm) at 35°C. The UPLC-PDA/QDa method used a gradient elution starting with 95% A (5mM ammonium formate (pH 3)) and 5% B (0.2% formic acid in acetonitrile) to 55% B at 0.4 mL/min using an Acquity® UPLC HSS (high strength silica) C18 1.8- $\mu$ m column (150 mm x 2.1 mm) at 50°C. A dilution series was analyzed to determine the limit of detection (LOD) for each method with the UPLC-PDA/QDa method giving a lower limit of detection (LLOD) of 5 ng/mL with a 3.0  $\mu$ L injection volume for all compounds studied. Complete resolution was obtained for 10 of the 12 tryptamines by HPLC-PDA. Only two compounds could not be differentiated by UV given their co-elution and similar UV spectra. Separation and differentiation, however, was achieved using the UPLC/PDA-QDa which combined the high efficiency of UPLC with PDA and MS technology. Comparison of the biphenyl and C18 columns resulted in similar elution order.

UPLC/PDA-QDa is an excellent complement to standard instrumentation as it provides both chromatographic and mass spectral information without analyte degradation, while being sensitive and accurate for tryptamine analysis.

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**UHPLC-PDA/QDa, Tryptamines, Hallucinogens**

## **B39 Community Resources and Updates from the Scientific Working Group for DNA Analysis Methods (SWGDM) Next Generation Sequencing (NGS) Committee**

*Katherine Gettings, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD*

**Learning Overview:** Attendees will learn about the activities of the SWGDAM NGS Committee, including currently available and forthcoming resources to support the adoption of new sequencing technologies for forensic DNA analysis.

**Impact Statement:** Impact to the forensic community is increased awareness of available resources; increased discussion will also impact future efforts of the committee.

The Scientific Working Group for DNA Analysis Methods (SWGDM) Next Generation Sequencing (NGS) Committee began as a working group in 2014 and formalized as a committee in 2018. This committee is tasked with identifying, evaluating, and researching issues relating to the forensic applications of next generation sequencing. On behalf of the committee members, this presentation will update the forensic community on past, current, and future efforts and work products. Past efforts include NGS-specific interpretation guidance for autosomal STR sequencing<sup>1</sup> and mitochondrial DNA analysis.<sup>1,2</sup>

Two recent efforts/resources will be described in detail: 1) SNP interpretation guidance and 2) STR sequence mixture data intended to support the development of probabilistic genotyping methods. The SNP interpretation guidance document is focused on commercially available SNP assays most likely to be implemented in a forensic DNA laboratory. These assays target identity, ancestry, and/or phenotype informative SNPs; however, guidance is currently lacking regarding interpretation, statistical analysis, and reporting of this marker type. This document represents a first step in collating existing information and standardizing implementation across laboratories. The STR sequence mixture data project is steered by the SWGDAM NGS committee, with mixture samples created and sequencing analysis performed by the NIST Applied Genetics Group. This mixture data set initially consists of 96 samples sequenced in several commercially available forensic STR sequencing assays. The mixture compositions were determined based upon feedback from probabilistic genotyping software developers. All mixture and single source contributor data are available in a NIST Public Data Repository.<sup>3</sup>

Future efforts of the SWGDAM NGS Committee include an NGS-specific validation guidance document and additional support for probabilistic genotyping and bioinformatic software developers.

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### **NGS, SWGDAM, DNA Sequencing**

## B40 Standards Development Activities in Human Forensic Biology

*Kristy Kadash, PhD\**, Jefferson County Regional Crime Lab, Golden, CO; *Beth Ordeman, MS*, Pinellas County Forensic Laboratory, Largo, FL

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards pertinent to the field of human forensic biology.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to human forensic biology. It will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards. During this presentation, updates related to standards development in Human Forensic Biology will be discussed. These include:

Recent standards that have been added to the OSAC Registry:

- **ANSI/ASB Standard 023**, *Standard for Training in Forensic DNA Isolation and Purification Methods, First Edition, 2020.*
- **ANSI/ASB Standard 110**, *Standards for Training in Forensic Serological Methods, First Edition, 2020.*
- **ANSI/ASB Standard 115**, *Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection, First Edition, 2020.*
- **ANSI/ASB Standard 116**, *Standard for Training in Forensic DNA Quantification Methods, First Edition, 2020.*
- **ANSI/ASB Standard 018**, *Standard for Validation of Probabilistic Genotyping Systems, First Edition, 2020.*

OSAC proposed standards:

- **OSAC 2020-S-0004**, *Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events.*
- **OSAC 2020-N-0007**, *Best Practice Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis.*

Published standards from the Academy Standards Board (ASB) pending Registry approval process:

- **ANSI/ASB Standard 038**, *Standard for Internal Validation of Forensic DNA Testing Methods, First Edition, 2020.*
- **ANSI/ASB Standard 077**, *Standard for the Developmental and Internal Validation of Forensic Serological Methods, First Edition, 2020.*
- **ANSI/ASB Standard 130**, *Standard for Training in Forensic DNA Amplification Methods for Subsequent Capillary Electrophoresis Sequencing, First Edition, 2021.*
- **ANSI/ASB Standard 131**, *Standard for Training in Forensic DNA Sequencing using Capillary Electrophoresis, First Edition, 2021.*

Documents currently in development at the OSAC or by the ASB:

- *9 training standards from the training standards suite*
- *Standard for Internal Validation of Human STR Profiling on CE Platforms (and BPR)*
- *Standards for Prevention, Monitoring, and Mitigation of DNA Contamination*
- *Assigning Propositions for Likelihood Ratios in Forensic DNA Interpretations*
- *Best Practice Recommendation for Validation of Forensic DNA Software*
- *Standard for Internal Evaluation of a Laboratory's DNA Mixture Interpretation Protocol*
- *Standard for Reporting DNA Conclusions*
- *Standards for Determining Analytical and Stochastic Thresholds for Application to Forensic DNA Casework Using Electrophoresis Platforms*
- *Standards for Validation and Implementation of Familial Searching for Forensic Purposes*
- *Forensic Autosomal STR DNA Statistical Analyses –General Protocol, Protocol Verification, and Case Record Requirements*
- *Best Practice Recommendations for Testimony Regarding DNA Activity Propositions*

Other highlights:

- Provided subcommittee response to the NIST Foundational Review on DNA Mixture Interpretation.
- Forensic Biology Process Map
- Technical Report: Appendix Examples for Biology/DNA Reports
- 4Pilot audit document for **ASB Standard 040** *Standard for Forensic DNA Interpretation and Comparison Protocols.*
- New research needs.

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

### Human Forensic Biology, Standards, OSAC

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## **B41 A Short Tandem Repeat (STR) Sequence Nomenclature Update From the Short Tandem Repeat: Align, Name, Define (STRAND) Working Group**

*Katherine Gettings, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD; *David Ballard, PhD*, Kings College, London, United Kingdom; *Martin Bodner, PhD*, Medical University of Innsbruck, Innsbruck, Austria; *Jonathan King, MS*, University of North Texas Health Science Center, Fort Worth, TX; *Walther Parson, PhD*, Medical University of Innsbruck, Innsbruck, Austria; *Christopher Phillips*, University of Santiago de Compostela, Santiago de Compostela, Spain

**Learning Overview:** Attendees will learn about the activities of the STRAND Working Group, including currently available and forthcoming resources on STR sequence nomenclature to support the adoption of STR sequencing technologies for forensic DNA analysis.

**Impact Statement:** Impact to the forensic community is increased awareness of available resources; increased discussion will also impact future efforts of this working group.

Since the 2016 publication of the ISFG DNA Commission Considerations on Minimal STR Sequence Nomenclature Requirements, the STRAND Working Group has been collaborating to harmonize related STR sequencing efforts across our respective laboratories: STRidER STR sequence quality control, STRSeq catalog of sequences, STRait Razor bioinformatic freeware, and the Forensic STR Sequence Structure Guide.<sup>1-5</sup> To expand and advance this discussion, in 2019 the STRAND working group hosted a meeting on STR sequence nomenclature with attendees across government, academic, and vendor sectors. Content from this meeting was disseminated to the community through publication and presentations.<sup>6</sup>

Following this meeting, an ISFG DNA Commission on STR Sequence Nomenclature was initiated in 2020 to develop recommendations for maintaining, formatting, and exchanging STR sequence data. These recommendations follow the tradition of autosomal STR allele designation guidance from earlier DNA Commissions of the ISFG which further evolved as the technology expanded into Y-STRs. The starting point for this DNA Commission was the 2016 *considerations* paper; now reevaluated in light of the intervening five years of published data and technology advancements.<sup>1</sup> This DNA Commission is composed of members of the STRAND working group and additional experts from international academic and government laboratories.

On behalf of the STRAND working group, this presentation will describe the aforementioned efforts of working group members, as well as the ISFG DNA Commission's progress toward STR sequence nomenclature recommendations covering: (1) sequence strings and minimum reporting range, (2) bracketed sequences, (3) resources for sequence formatting, (4) characterizing new loci, and (5) interlaboratory data exchange.

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### **STR, Sequencing, Nomenclature**

## B42 An Evaluation of the Current Opinion of Next Generation Sequencing (NGS) Technology in Forensics Through a Global Survey

Megan Foley, MSFS\*, Center for Forensic Science Research & Education, Willow Grove, PA and George Washington University, Washington, DC;  
Fabio Oldoni, PhD, Arcadia University, Glenside, PA

**Learning Overview:** After attending this presentation, attendees will better understand the current status of next-generation sequencing (NGS) in crime laboratories around the world and the opinions of forensic DNA researchers/practitioners on the issues and future implementation of NGS in casework.

**Impact Statement:** This presentation will impact the forensic science community by exploring the opportunities for improvement and needs for implementation of NGS technologies into research and casework laboratories based on survey data.

Next-generation sequencing (NGS), also known as massively parallel sequencing (MPS), is gradually gaining traction in the forensic field as a few crime laboratories are beginning to validate and implement this new technology in casework.<sup>1,2</sup> Forensically relevant research on NGS has been ongoing in the field and an increasing volume of data has been published that focuses on furthering the amount of information that can be generated from genetic material collected from crime scene evidence.<sup>3,4</sup> However, the implementation and acceptance in the overall forensic community on the use of NGS on actual casework has been slow, especially in the United States. NGS data allows informative genetic information to be generated beyond traditional capillary electrophoresis (CE) practices that are widely used and accepted in the global community. Due to its enhanced multiplexing capability, hundreds of genetic markers can be targeted and processed simultaneously. Furthermore, individual sequences of STR markers are determined which enhances deconvolution of multiple contributors in a mixed profile or separation of true allelic peaks from artifactual data. Additional information can be obtained through the analysis of large SNP assays that can provide an analyst with more identifying information for kinship testing or predict physical traits through ancestral or phenotypic biomarkers.<sup>5,6</sup>

This study was distributed to over 5,000 forensic DNA researchers and practitioners who are current members of the International Society for Forensic Genetics and the American Academy of Forensic Science through a Qualtrics survey. Data were collected from a 20-question online survey hosted by Arcadia University and The Center for Forensic Science Research and Education and approved by the Arcadia's IRB board. Questions included location and information about the laboratory of the responder as well as training/experience received in addition to current NGS technology applications, future technology usage, and opinions of issues of utilizing NGS in the field. The survey had a total of 367 respondents. The majority of respondents were from North and South America (69.75%), followed by Europe (21.25%), Asia (5.45%), Oceania (2.45%), and Africa (1.09%). A total of 187 respondents were practitioners, 115 researchers, and 65 performed both research and casework. About 38% of the responders currently have an NGS system in place in their laboratory and about 13% are planning to purchase an NGS instrument.

The results of the survey indicate that there is general knowledge of the application of NGS in forensics. Nonetheless, some of the major hurdles that many laboratories are presently facing in order to implement NGS into their analytical workflows include among others funding, staffing, limited value added, time and cost-effectiveness. Additionally, there are several technical concerns that respondents have even if they had the time and funding in their laboratory to purchase a massively parallel sequencing instrument. Some of the main concerns included training, no current statistical applications, bioinformatics support, lack of well-defined recommendations and guidelines, and court admissibility. Overall, the data displays that the respondents believe there will be a technology shift from CE to NGS in the next 5-10 years. In a nutshell, this presentation will illustrate the current usage of NGS technology throughout the world and will address strategies to overcome current analytical challenges for its implementation.

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### Next Generation Sequencing (NGS), Online Survey, Forensic DNA

## B43 American Forensic Practitioners' Opinions on Activity-Level DNA Reporting

Yoon Yang, MS\*, Arcadia University, Glenside, PA; Mechthild Prinz, PhD, John Jay College of Criminal Justice, New York, NY; Heather McKiernan, PhD, Center for Forensic Science and Research Education, Willow Grove, PA; Fabio Oldoni, PhD, Arcadia University, Glenside, PA

**Learning Overview:** By attending this presentation, attendees will have a better understanding of the status of activity level evaluative reporting in the United States.

**Impact Statement:** This presentation will impact the forensic science community by serving as a gap analysis of this area of research and give insight to any differing opinions among forensic practitioners in the U.S.

In criminal cases involving DNA evidence, it may not be the source of the DNA that is disputed in court, but rather how the DNA was deposited at the scene of crime.<sup>1-3</sup> Activity level propositions have been considered to address and answer this question.<sup>1-3</sup> However, there are many factors that should be included when formulating propositions at activity level, such as transfer, persistence, prevalence, and recovery (TPPR). Nevertheless, European laboratories have used evaluative reports under the hierarchy of propositions in court, and the European Network of Forensic Science Institutes (ENFSI) has issued specific DNA guidelines on the process. Despite this, the overall views of activity level DNA reporting in the U.S are not well known. Therefore, this study aimed at obtaining an overview on the opinions of using activity level reporting held by forensic DNA practitioners in the United States.

A seventeen-question survey was distributed through Qualtrics™ to members of the American Society of Crime Laboratory Directors (ASCLD) via the weekly Crime Minute and the International Society for Forensic Genetics (US members only) via email. The survey included multiple choice and open response questions and reached around 650 people.

Overall, there were 54 respondents to the survey in which 44 of them had over ten years of experience in DNA reporting at activity level. Of those 44 with over 10 years of experience, 72% agreed that despite having some concerns activity level reporting would be very (27%) or moderately (45%) useful. Only 31% believed that the current studies on DNA transfer were moderately adequate to provide empirical information on a case. A total of 41% of participants agreed that one year of training on activity level DNA reporting would be sufficient for a scientist to testify as an expert witness. There were six major concerns for implementing activity level DNA reporting in the U.S.: (1) the number of variables to be considered in activity level reporting such as shedder status, amount of starting DNA, or differing transfer rates based on surfaces; (2) educating practitioners and the legal system; (3) lack of controlled studies with realistic scenarios; (4) issues in court with admissibility. With this regard, some participants expressed concern that activity level propositions will have a difficult time passing a Daubert hearing because the approach is not as concrete or statistically sound as a DNA profile match. Moreover, (5) need for a standardized approach or guidelines in the U.S.; and (6) convincing the forensic community and reaching consensus.

These concerns expressed by U.S. forensic practitioners revealed a range of varying opinions on activity level reporting. Future research will involve expanding the participants to a global level.

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### Forensic Science, DNA Transfer, Activity Level Propositions

## B44 Microbial Dynamics of Human Decomposition Accurately Estimate Postmortem Interval

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding on the importance of studying the microbiome associated with human decomposition for forensic purposes.

**Impact Statement:** This presentation will impact the forensic science community by conveying how the microbial response to human remains can be modeled to estimate the postmortem interval.

Nearly 64,000 cases (42%) of homicide and non-negligent manslaughter went unsolved in the United States from 2010 to 2019.<sup>1</sup> One approach to solving these cases is to accurately estimate the postmortem interval (PMI), or time since death, using decomposition processes. Estimating PMI can help solve death investigations because it can be used to identify people and establish the investigative timeline. One form of physical evidence that is always associated with decomposing remains is the postmortem microbiome. The postmortem microbiome has predictable characteristics that have proven to be useful for estimating PMI.<sup>2</sup>

This study provides the first analysis in using multiple ‘omic techniques for the accurate estimation of postmortem interval, leveraging three anthropological facilities to place 36 human bodies to decompose outdoors over multiple seasons. This study used amplicon DNA, metagenomic DNA, and metabolite datasets to build machine learning models with the goal of using ‘omic data feature abundance and geographic location as predictors of PMI. These results show that model accuracy was dependent on taxonomic resolution and site where the sample was derived. The models generated from 16S rRNA genes isolated from the corpse skin and classified at the species level provided the most accurate predictive power over the first 21 days of decomposition. Interestingly, in the gravesoil, taxonomic abundances predicted PMI more accurately than functional features (e.g., gene and metabolite abundances) suggesting either community structure correlates best with decomposition time or higher resolution is required for other ‘omic data types. Further, microbial abundance was more important for predicting PMI than geographic location suggesting shared trends between the three anthropological facilities exists which allow for the use of cross-facility models. This study shows that accurate, microbially-based models for aiding in the estimation of PMI for death investigations can be obtained from multi-omic data including cost-effective, amplicon sequencing data.

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### Postmortem Interval (PMI), Microbiome, Decomposition

## **B45 A Pilot Study of Microbial Succession in Human Rib Skeletal Remains During Terrestrial Decomposition**

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**Learning Overview:** After attending this presentation, attendees will learn about microbially-mediated decomposition of skeletal remains in a terrestrial environment. They will also learn how technologies (high throughput DNA sequencing of microbial communities) can be used to estimate the postmortem interval (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 Statement:** This presentation will provide the forensic science community with a better understanding of novel microbiome tools that may be useful in investigations of unattended death scenes, in which PMI is unknown. In particular, this presentation will show how this approach can be applied to death scenes that are several weeks or months old in which only skeletal remains are found.

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 three 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 if microbial succession is similar 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.” We hypothesize 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, we characterized the human bone microbial decomposer community to determine whether microbial succession is a marker for PMI. Six human donor subjects were placed outdoors to decompose on the soil surface at the Southeast Texas Applied Forensic Science facility. To also assess the effect of seasons, three decedents were placed each in the spring and summer. Once ribs were exposed through natural decomposition, a rib was collected from each body for 8 time points at 3 weeks apart for a total of 48 sampled ribs. 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 UV irradiation, then pulverizing 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.

We discovered a core bone decomposer microbiome dominated by taxa in the phylum Proteobacteria and evidence that these bone-invading microbes are likely sourced from the surrounding decomposition environment, including skin of the cadaver and soils. Additionally, we found significant overall differences in bone microbial community composition between seasons. Finally, we used the microbial community data to develop random forest models that predict PMI with an accuracy of approximately 34 days over a 1- to 9-month time frame of decomposition. Typically, anthropologists provide PMI estimates based on qualitative information, giving PMI errors ranging from several months to years. Previous work has only focused on the characterization of the bone microbiome decomposer community, and this is the first known data-driven, quantitative PMI estimate of terrestrially decomposed human skeletal remains using microbial abundance information.

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### **Bone, Microbiome, Postmortem Interval (PMI)**



## B46 Forensic Microbiology of Human Cadavers in an Experimental Mass Grave

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**Learning Overview:** Attendees will learn about the impact of freezing and thawing on the postmortem human microbiome, and the implications this may have for experimental microbiome studies at human taphonomy facilities. Additionally, they will learn about metabarcoding experiments currently taking place as part of the Mass Grave Project and about their expected impact on the understanding of microbial successions over the course of time in an experimental mass grave context.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the validity of microbiome analyses using donated bodies in simulated mass graves and the importance of routinely collecting microbiome samples from body donations prior to their freezing and/or deposition to maximize the chances of obtaining relevant microbiome results for the forensic population.

Microbiome studies are attracting increasing attention among forensic experts due to their potential to reveal valuable information related to individual identity, including geographical origin, health status, postmortem interval (PMI), and even lifestyle of the deceased.<sup>1</sup> To date, microbiome studies have mostly used animal proxies, allowing for large samples to increase statistical power of results.<sup>2</sup> However, to validate microbiome-based methods for application in forensic casework, further research on human cadavers is needed. Metabarcoding studies are currently being conducted on individual buried or exposed cadavers at human taphonomy facilities across the world, with the aim to map the microbial shifts associated with gross decompositional changes.<sup>3-5</sup> It has been previously shown that microbial populations are still altered after four years post the deposition of multiple cadavers in a shallow burial.<sup>6</sup> However, microbial shifts have not yet been studied via metabarcoding in the context of a mass grave, where the presence of multiple decomposing bodies could significantly alter the composition and development of the thanatomicrobiome. Additionally, it is relatively common for donated cadavers to be frozen prior to human decomposition studies, particularly when placement of multiple cadavers simultaneously is required. Previous studies have demonstrated that freezing and thawing can alter the thanatomicrobiome, but there is currently no consensus on the degree of alteration and/or the longer-term effects on its development throughout decomposition.

This project implements metabarcoding analyses on cadavers and soil samples in the first mass grave experiment conducted at Texas State University's Forensic Anthropology Research Facility (FARF). The Mass Grave Project includes a mass grave with six donated human cadavers, a control mass grave without cadavers, three individual graves with cadavers, and one (empty) individual control grave, all buried in May 2021. The experiments will be monitored for 18 months, after which the graves will be excavated. Human microbiome samples were collected by swabbing five different locations on the body (hand, foot, neck, mouth and rectum) prior to freezing, after thawing/before burial, and further samples will be collected upon excavation. The aims of this study are to: (1) examine the effect freezing donated cadavers has on microbiome sampling in taphonomic studies; (2) examine microbial shifts associated with increasing PMIs in a mass grave, how they differ from single burials and how they migrate within different areas of the grave; and (3) compare the microbiome of different body sites before and after the decomposition, to identify good microbial indicators that could be used for estimating prolonged PMIs.

Soil samples are being collected from the graves throughout the 18-month duration. Microbial DNA will be extracted using Qiagen DNeasy PowerSoil Pro Kit and will be analyzed via sequencing of the V4 region of the 16s rRNA gene using Illumina MiSeq platform. NGS data will be analyzed via R scripts normally used in metabarcoding ecology studies. The results on the first aim of the study will be presented and guidelines on microbiota sampling of willed body donations will be proposed for implementation in human taphonomy facilities worldwide.

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### (Thanato)microbiome, Mass Grave, PMI

## B47 Bone Fluorescence, Taphonomy, and the Postmortem Interval (PMI)

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**Learning Overview:** After attending this presentation, attendees will understand the influence of the decomposition microenvironment on the degradation of organic bone and subsequently, the limited potential of using alternate light sources (ALS) to determine general postmortem interval (PMI).

**Impact Statement:** This presentation will impact the forensic science community by challenging the use of bone fluorescence as a general indicator of PMI and provides experimental data suggesting that the decomposition microenvironment is too variable to allow for bone fluorescence to act as a reliable indicator of forensic relevance.

Bone fluoresces under short-wave visible light due to the presence of fluorophore amino acids in collagen. Phenylalanine, tryptophan, and tyrosine, all present in the organic portion of human bone, have aromatic side ring chains, allowing these amino acids to be optically active under short-wave visible light.<sup>1</sup> Postmortem, however, proteins containing these fluorophore amino acids degrade, subsequently causing a decrease in the fluorescence intensity of bone.<sup>2</sup> This has catalyzed interest in using fluorescence intensity as an indicator of general PMI.<sup>2</sup>

To test the potential use of fluorescence as a PMI indicator in an arid environment, the skeletons of eleven individuals placed in the outdoor facility at the Forensic Investigation Research Station (FIRS) in Whitewater, Colorado were examined. All individuals had a known PMI and remained outdoors between 101 and 1,876 days. Following their removal from the outdoor facility, all were cleaned using warm-water maceration, and stored in the FIRS Donated Human Skeletal Collection. On-site weather data was also incorporated into this study to determine if variables other than time had an influence on bone fluorescence. Bones from each individual were photographed under a 430-470 nm Crime-lite with the use of an orange filter. The photographs were then analyzed in ImageJ to determine the intensity of fluorescence, following a modified version of the methodology outlined by Swaraldahab and Christensen.<sup>2</sup>

All individuals included in this study displayed fluorescence values lower than the ancient samples analyzed by Swaraldahab and Christensen.<sup>2</sup> The single bone displaying the highest fluorescence intensity value was approximately equivalent to the ancient average described by Swaraldahab and Christensen, while the single bone displaying the lowest fluorescence intensity value was barely visible under the ALS.<sup>2</sup> Even when ambient temperature and solar radiation were considered in addition to time, no correlation existed between any single variable and bone fluorescence. Therefore, fluorophore degradation in collagen is not linear with respect to any single variable and likely depends on several factors, as it cannot be explained by UV exposure, ambient temperature, or time alone.

Furthermore, caution should be used when employing ALS searches in a forensic context, as suggested by some authors. Forensically relevant skeletal elements may not fluoresce even after a comparatively short time period, and in some environments, are unlikely to fluoresce at all. When an ALS search is used, a lack of discernable fluorescence should never be taken to mean that no bone material is present.

The results of this study highlight the importance of considering regional taphonomic processes in any skeletal study. Methodologies of estimating PMI, even on a general scale, may prove useful to buried populations or those in more temperate environments, but fail when introduced into more extreme climates. Bone fluorescence should only ever be used during ALS searches in a supplementary way and should never be used as an estimate of PMI due to its extreme variability.

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### Taphonomy , Alternate Light Source , Fluorescence

## B48 Bone “Soft” Maceration Procedures at Human Taphonomy Facilities: How “Soft” Are They Really?

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**Learning Overview:** Attendees will understand the impact that different “soft” maceration processes commonly used by human taphonomy facilities for treating bones prior their inclusion in skeletal reference collections have on bone structure and on bone biomolecules, and how these procedures limit potential microscopic and molecular analyses of bone for in vivo and postmortem ageing studies.

**Impact Statement:** This presentation will impact the forensic science community in terms of competence, by showcasing the effects that “soft” maceration techniques have on bone biomolecules and on bone mineral structure, and performance, highlighting the potential biases caused by bone processing treatments can be avoided if alternative methods are sought by the community.

Bones are a precious reservoir of information on the identity of the deceased. Traditional morphological and metric anthropology approaches have been largely used for biological profiling of unknown skeletal remains.<sup>1</sup> In case of poor preservation, fragmentation or incompleteness of the skeletal remains, microscopic and molecular techniques represent important tools to aid human identification. Biomolecular and microscopic techniques can lead to individual identification via DNA analysis, or reveal information on age at death (AAD) and PMI using proteomics, metabolomics/lipidomics, and biophysical analysis.<sup>2-6</sup> To successfully extract biomolecules without inducing decay, and to avoid the introduction of biases in data interpretation, the structural and molecular composition of bones must remain unaltered by thermal, chemical or mechanical treatments. However, in many human taphonomy facilities, bones undergo several cleaning steps, including “soft” maceration processes prior to long term storage, in order to remove remaining soft tissues after cadaveric decomposition. Depending on the harshness of the methods used, bone physicochemical and molecular properties can be moderately to severely impacted, resulting in a significant loss of information and limiting the usability of these remains in microscopic and molecular studies.

To comprehensively evaluate the extent of damage caused by maceration to bone biomolecules and biophysical properties, three complete right bovine tibiae were experimentally macerated using three different protocols routinely performed at Forensic Anthropological Center at Texas State (FACTS) (n=1) and at Southeast Texas Applied Forensic Science (STAFS) (n=2). These protocols differ by duration of the treatment, water temperature, and cleaning agents used. As part of the “ForensOMICS” project, DNA, proteins, lipids and metabolites were extracted and subjected to sample quality control (DNA) and full omics analysis (proteomics, lipidomics and metabolomics). In addition, a combination of Fourier-transform infrared spectrometry based on attenuated reflectance mode (ATR) and ATR microscopy were performed, to gain insight into mineral degradation induced by the three different treatment protocols. Results from macerated bones were compared to the untreated left tibiae, to take into account the existing biological variability among different animals.

A significant reduction in protein abundances and variety was observed in macerated bones in comparison with the untreated counterparts, particularly when using the protocol with the warmest water temperature. Similarly, metabolites and lipids showed a severe reduction in abundance and revealed a contamination caused by cleaning agents. Finally, mineral crystallinity and phosphate content were heavily affected by treatments, especially when high temperature was applied.

Overall, these findings suggest that caution should be taken when interpreting biomolecular and biophysical results from macerated bones, particularly when data are used to produce regression models for AAD and PMI estimation. Submerging bones at high temperatures (>60°C) for short times is more damaging than a prolonged submersion at lower temperatures, as it heavily affects mineral and organic bone matrix. Ideally, cleaning and maceration protocols should be standardized across human taphonomy facilities, to maximize the data comparability and facilitate comparative studies between taphonomic collections from different environments. Minimally damaging and invasive protocols are needed in order to prepare skeletal remains for long term storage and continued scientific analyses.

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### Bone Maceration, Bone Biomolecules, Bone Biophysics

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## **B49 The Optimization of the InnoXtract™ Extraction and Purification System for DNA Extraction From Skeletal Samples and Analysis of Subsequent Downstream Processing Methods**

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**Learning Overview:** This presentation will provide an overview of the optimization process of DNA extraction and purification from skeletal samples using InnoXtract™ methods. Additionally, a method for assessing which downstream process is better suited for skeletal extracts (on a case-by-case basis based on the quality and quantity of the DNA extract) will be examined.

**Impact Statement:** Improving human identification methods is an ongoing concern. The introduction of a new DNA extraction and purification method suited for challenging skeletal samples can improve the quantity and quality of DNA recovered. By analyzing a variety of extracts using STR typing, microarray SNP typing, and massively parallel sequencing methods, a procedure for assessing which downstream process is best suited based on the quantity and quality of the extract can be determined. This can provide analysts with a more streamlined process, limit the loss of DNA extract to unsuitable methods, and allow for the implementation of investigative methods such as genetic genealogy or phenotype/ancestry prediction methods.

Often, only skeletal remains are available in human identification cases, requiring challenging DNA typing methods on these samples for definitive identifications. The low-template nature, prevalence of inhibitors, and fragmentation commonly found in skeletal samples complicates this task of human identification through DNA typing and requires additional research on suitable DNA extraction, purification, and downstream processing techniques.<sup>1,2</sup>

InnoXtract™ (InnoGenomics Technologies) extraction and purification system designed for DNA extraction from low-template samples, specifically rootless hair shafts.<sup>3</sup> In addition, its ability to capture highly fragmented DNA suggested its suitability for use with skeletal samples<sup>3</sup>. However, the lysis and digestion parameters required modifications to optimize the method for this sample type.

Preliminary studies determined that InnoXtract™ purification parameters were most compatible with PrepFiler™ BTA (Thermo Fisher Scientific) lysis parameters; therefore, InnoXtract™ lysis parameters were designed similarly to this method. A two-part digestion was developed using a homebrew digestion buffer (0.5M EDTA, 0.05% Tween 20, and 100 mM NaCl) and a supplemental lysis with the hair digestion buffer included in the InnoXtract™ kit. The InnoXtract™ purification parameters were also modified to better suit skeletal samples by increasing the volume of magnetic beads allowing for maximal capture of DNA fragments. Following these modifications, the optimal volume of sample input was examined. The optimized InnoXtract™ method provided extracts with similar quality and quantity of recovered DNA compared to the commercial skeletal extraction method, PrepFiler™ BTA.

The optimized InnoXtract™ and PrepFiler™ BTA extraction methods were performed on various challenging skeletal samples (from surface decomposed, buried, embalmed, burned, and cremated remains). These extracts will be analyzed using three downstream processing methods; STR typing (Investigator 24plex QS; QIAGEN), microarray SNP typing (Infinium Global Screening Array; Illumina Inc.), and massively parallel sequencing techniques (ForenSeq Signature Prep Kit and ForenSeq Kintelligence Kit; Verogen). By examining the overall success of each method compared to each extract's DNA quantity/quality, a recommendation on the best-suited method will be determined. Initial data indicates several extracts have sufficient DNA recovery to yield complete STR profiles. Additionally, the Global Screening Array demonstrated sensitivity for DNA input down to 200 pg, indicating the improved sensitivity for forensically relevant SNPs. The ForenSeq Kintelligence kit provided an average total call rate of 9938 ± 258 SNPs at 1 ng input from challenging skeletal remains. These data indicate the potential success of this new extraction method with compromised skeletal remains utilizing a variety of downstream processing techniques.

This presentation will highlight the success of the newly optimized InnoXtract™ method for DNA extraction and purification from skeletal samples and demonstrate what downstream process should be implemented depending on each extract's DNA quantity and quality.

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### **Skeletal Remains, Human Identification, DNA Extraction**

## **B50 The Application of Ancient DNA Methodologies to Burned Forensic Samples and Their Potential to Aid in the Identification and Analyses of Difficult Samples**

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**Learning Overview:** The purpose of this presentation is to present a generalized overview of the experimental design and preliminary data related to the potential gains expected from incorporating methodologies adopted from the analyses of ancient DNA regarding the efficacy of the isolation and analysis of degraded DNA from badly burned forensic samples.

**Impact Statement:** After completion, this research will provide a much-needed generalized set of suggested protocols for the isolation and analyses of burned forensic samples. This research will serve as an optimal sampling guide for basic sampling strategies and locations, DNA extraction methods, short tandem repeat (STR) profiling, and mitochondrial DNA (mtDNA) library preparation when dealing with degraded or burned forensic samples.

The ability to identify human remains using DNA analyses has revolutionized forensic science and become a standard procedure. However, most analyses generally rely on the presence of ample, minimally degraded endogenous DNA, which may not always be available. This is especially true when considering source material which may have compromised DNA retention due a variety of factors, including post-mortem decay and other alterations such as burning (thermal) and insect activity etc.

For temporally degraded remains, the breakdown of DNA molecules over time can drastically reduce DNA yields, DNA fragment length, and/or alter base composition via the conversion of cytosine to uracil through hydrolysis. As a result, the field of ancient DNA (aDNA) analyses has independently developed protocols for sampling, DNA extraction, and analyses optimized to take this degradation into account and maximize the quantity and quality of endogenous DNA recovered. In forensic DNA analysis, recent studies have shown that the adoption of aDNA techniques can lead to more successful outcomes in forensic samples having undergone minimal temporal degradation.

However, not all sample degradation is due to time or other environmental factors. The exposure of tissue to extreme temperatures, such as when burned, similarly affects DNA degradation in that much of the DNA is destroyed outright. Furthermore, the fragment length of recovered DNA may be drastically shorter, depending on the level of thermal exposure. Currently, the standard measure of the thermal alteration of remains is categorized based on severity along a scale of one to five. Tissue exhibiting low levels of alteration (e.g. superficial charring) being graded as a “one,” and the most severely altered tissues being graded at level “five” (fully burned cremains). Previous studies have shown that DNA recovery becomes increasingly difficult as the burn category of remains increases. The efficacy of aDNA techniques has only just begun to be explored. However, preliminary studies show that they may be more efficient than modern techniques, especially in regards to heavily altered samples in burn categories four and five. Presented here is a continuation of this research focusing on samples in burn categories three and higher, comparing forensic and ancient DNA laboratory protocols including the previously studied Dabney ancient DNA extraction protocol vs. the Lorielle modern forensic protocol as well as a new comparison of single-stranded next-generation sequencing (NGS) library preparation vs. double-stranded library techniques. In addition, a systematic analysis of DNA yields across a wide range of skeletal elements and associated sampling locations will serve as a basis for future sampling strategy formation.

Ten donor cadavers were burned under similar conditions at the University of Tennessee Research Center. From each of these individuals, the same twelve skeletal elements exhibiting the characteristics of burn categories three or higher were then collected and sent to Arizona State University’s School of Human Evolution and Social Change (SHESC) for DNA extraction and analyses. DNA extractions using both tested protocols are performed using the same source material for each sample. The DNA extracts are then analysed for both DNA yield and STR profile recovery. Next, each extraction will then be converted into both double and singlestranded DNA sequencing libraries and be enriched for both mtDNA and a set of diagnostic single nucleotide polymorphisms (SNPs) for further analysis. Casework samples from the Office of the Medical Examiner, Maricopa County, Arizona, are also processed alongside the controlled samples to establish a better sense of practical expectations. This study will serve as an optimal sampling guide for basic sampling strategies and locations, DNA extraction methods, STR profiling, and target enriched DNA library preparation from degraded or burned samples.

### **Forensic DNA, Method Development, Burned Remains**

## B51 Rapid DNA Profiling for Disaster Victim Identification (DVI)

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**Learning Overview:** Attendees can expect to learn about rapid DNA profiling protocols for decomposed human remains and disaster victim identification (DVI).

**Impact Statement:** This presentation has the potential to expedite the disaster victim identification (DVI) process, especially in remote locations.

Advances in forensic biology have increased options for the collection, sampling, preservation and processing of human remains for DNA-based identification. Targeting minimally-invasive post-mortem (PM) sample types that are simple to collect and do not require extensive preparation and testing protocols such as nail, distal phalanges (including whole digits) and femur drillings, is useful for DVI with sampling amenable to in-field collection.<sup>1-3</sup> Approaches which preserve sample, reduce analytical turnaround times and save resources, have the potential to expedite the identification process and provide answers to grieving families sooner.

At the Australian Facility for Taphonomic Experimental Research (AFTER), a human taphonomic facility, authentic PM samples in various stages of decomposition were collected from human cadavers and subjected to rapid collection, preparation and processing applications. A range of environmental conditions and deposition states were considered including surface and sub-surface decomposition, with post-mortem intervals (PMI) ranging from zero days to four years.

In 2019 and 2020, DVI simulations were conducted at AFTER. Whole and fragmented human cadavers were positioned to replicate a building collapse scenario and left to decompose for up to four weeks. This study evaluated the utility of the ANDE™ 6C Rapid DNA System and the RapidHIT™ ID System for DVI in the field and mortuary.<sup>2</sup> Applying PM nail and tissue biopsy samples showed promise, with the added benefit of minimally-invasive collection procedures and limited preparation requirements. Both platforms demonstrated utility for assisting with in-field identification and re-association of human remains.

Efficient DNA profiling approaches to PM samples were also developed for DNA-based identification. Incubating nail, distal phalanges and whole digits in 500 µL of PrepFiler™ Lysis Buffer for two hours was an efficient and simple method, limiting or removing sample preparation. A reduced 15-minute incubation also yielded DNA profiles, suggesting a shorter incubation may lyse sufficient DNA. These protocols have combined and modified previous work to develop a powder-free, rapid 15-minute PrepFiler™ Lysis Buffer incubation that is compatible with downstream automated processing; reducing sample preparation and extraction to two hours.<sup>3-5</sup> Immersing whole digits in leaching and preserving solutions such as dimethyl-sulfoxide-ethylenediaminetetraacetic acid-salt saturated (DESS) solution is also a viable option for DVI.<sup>3</sup> While the application of leached and preserved DNA to direct PCR on automated platforms was met with limited success<sup>6</sup>, swabs immersed in DESS were successfully applied to existing automated processes in an operational forensic laboratory.<sup>3</sup>

Optimal sample selection and collection can assist in facilitating downstream processing and can be combined with efficient DNA profiling protocols that involve minimal preparation and pre-treatment steps to expedite the identification of the deceased in mass fatality incidents. Furthermore, these protocols are compatible with existing DNA assays and automated instruments.

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### Taphonomy, Rapid DNA, Disaster Victim Identification

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## **B52 The Identification and Characterization of Viral Biomarkers**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the development of the human skin virome and its potential use as an alternative genetic marker. This study presents research identifying human skin viral markers that have both stability and sufficient diversity across the study population to generate a profile that is appropriate for forensic human identification.

**Impact Statement:** The goal of this presentation is to demonstrate the applicability and development of human skin virome biomarkers that can be utilized in an accurate, reproducible, and highly discriminatory pattern profile for human identification. In situations of very low DNA template availability, degradation of DNA can lead to stochastic amplification and incomplete allele detection. As such, in situations where traditional short tandem repeat (STR) analysis of human DNA is not possible, novel methodologies and tools need to be developed to utilize alternative, stable, and robust genetic sources for human identification. The human virome is a stable source of rich genetic diversity that has the potential to be utilized as one of these alternative genetic sources. Previous studies have explored the skin bacterial microbiome for forensic application; however there is a lack of studies into the human skin virome. The study described in this presentation evaluated viral signatures for human identification.

In this study, human skin viromes of 42 study participants consisting of three anatomical skin locations (right and left hand and scalp) were evaluated over a 6-month period ( $n=652$ ). Extracted skin virome samples were sequenced using the Illumina HiSeq 2500 platform to obtain a minimum depth of 12,000,000 reads per sample. The subsequent sequencing data was quality trimmed and filtered to remove low quality reads and Phi X contamination. All samples were mapped to the human genome to remove sequences of human origin. The resulting sequences were assembled on an individual sample basis. Additionally, all samples were pooled and assembled as a single dataset to improve assembly and annotation of sequences. The contigs generated were evaluated for viral gene presence and annotated using viral databases and were mapped back to each individual sample to measure viral presence and abundance. Statistical analysis was performed on annotated viral contigs and their abundance using relevant peer reviewed ecological statistic packages in R to assess virome stability, viral populations in the human skin virome, and to identify markers for human identification.

In total, 59 viral makers (38 viral species and 21 novel viral contigs) were identified as being significantly stable across four out of the five time points ( $P=5.3 \times 10^{-15}$ ) within subjects in the study population, using both database and metagenome assembly dependent and independent methodologies. All identified makers displayed stability across the three anatomical skin locations tested within at least one individual. The diversity profile of these identified markers was significantly different between individuals on the basis of presence and absence of the makers as measured by using binary Jaccard dissimilarity distances between all subjects ( $P=0.002$ ). Of these markers, seven of the identified viral species markers were present across the majority of samples and are thus proposed for future studies into genetic variation and single nucleotide polymorphisms comparisons for subject differentiation. Additionally, 21 of the established markers were novel viral genomes. This was determined by their genome containing at least one known viral gene. However, they had little to no sequence similarity to any viral or organism in current genome databases. The remaining 38 makers were all taxonomically annotated and were classified under the viral order *Caudovirales* and the viral families *Papillomaviridae*, *Genomoviridae*, and *Baculoviridae*. This presentation will discuss the utilization of the human skin virome for human identification purposes and introduce the development of an alternative genetic profile consisting of viral markers that can be used in conjunction with traditional human STR analysis to increase power of discrimination in resulting subject profiles.

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### **Virome, Biomarker, Human Identification**

## B53 Seasonal Comparisons Between Soil Fungal and Bacterial Communities Impacted by Human Decomposition

Lois Taylor, PhD\*, University of Tennessee, Knoxville, TN; Allison Mason, BS, University of Tennessee, Knoxville, TN; Jennifer DeBruyn, PhD, University of Tennessee, Knoxville, TN

**Learning Overview:** After attending this presentation, attendees will gain an understanding of seasonal (warm vs. cool season) fungal and bacterial successional patterns, how those patterns relate to prevailing soil chemistry during human decomposition over the long term (1 year), and their collective importance for time-since-death estimations.

**Impact Statement:** This work improves our understanding of both seasonal and long-term microbial community shifts observed during human decomposition and has implications for postmortem interval (PMI) estimation.

Terrestrial human decomposition creates localized hotspots of increased chemical activity, nutrient cycling, and microbial activity within the soil.<sup>1,2</sup> While microbial communities (bacterial and fungal) have been evaluated during warm-weather decomposition experiments, few studies have evaluated patterns associated with cool weather when insects are less abundant or absent. Furthermore, there has been limited comparison between bacterial and fungal successional characteristics across seasons, and limited associations with changes in soil chemistry. Bacterial and fungal decomposers utilize different resource pools and respond to altered nutrient and environmental (e.g., soil chemistry) conditions.<sup>3</sup> Since soil chemistry and nutrient profiles change dramatically during the decomposition process, this highlights a need for comparative study of these effects on bacterial and fungal communities as well as evaluating these changes by season.

The goals of this study were to reveal seasonal patterns in bacterial and fungal community structure during long-term decomposition (1 year) and to relate these changes to soil chemistry. Two year-long seasonal human decomposition trials were conducted at the University of Tennessee Anthropology Research Facility. A total of six donated human subjects (n=3 spring, n=3 winter) were placed at the facility and allowed to naturally decompose on the soil surface. Soil was subsampled at two depths: 0-1 cm (interface sample), and at 1-15 cm (core sample). 20 samples were taken annually for the spring study, and 19 for the winter study. Illumina amplicon sequencing was performed targeting the 16S rRNA and ITS2 gene regions in order to evaluate changes in bacterial and fungal communities. Soil chemical parameters measured included: pH, electrical conductivity (EC), respiration rates (evolved CO<sub>2</sub>), dissolved oxygen (O<sub>2</sub>) percentages, total carbon and nitrogen (TC/TN), and inorganic nitrogen concentrations (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>).

Principal coordinates analyses (PCoA) showed that both bacterial and fungal community composition significantly changed over time and differed by morphological decomposition stage in both seasonal trials (PERMANOVA,  $p < 0.05$ ). Irrespective of season, fungal community composition shifts exhibited two distinct inflection points coupled with changes in soil chemistry. The first occurred during the onset of advanced decay, corresponding to initial decreases in soil oxygen and pH, and increased NH<sub>4</sub><sup>+</sup>, EC, and CO<sub>2</sub>. The second occurred during late advanced decay, corresponding to recovery of soil oxygen levels to >75%, decreased NH<sub>4</sub><sup>+</sup>, and elevated NO<sub>3</sub><sup>-</sup> concentrations. Bacterial communities exhibited less clearly-delineated compositional shifts and higher variability (beta dispersion) during active and advanced decay. Additionally, bacterial communities stratified during late advanced decay, showing distinct community differences between cores and interface samples which remained differentiated at the end of the year-long study. Collectively, bacterial and fungal communities appeared to exhibit different successional driving mechanisms; while both responded to soil chemistry changes, fungal succession appeared more closely coupled with soil oxygenation. The magnitude of changes in soil chemistry parameters and microbial community structure was more pronounced in the spring study than in the winter study, as evidenced by a shorter-duration nutrient pulse with higher concentrations, and greater shifts in relative abundances of microbial taxa. After 1 year, soil communities remained altered and had not returned to their initial structure, regardless of season.

*Funding for this project was provided by the National Institute of Justice GRF-STEM (2017-R2-CX-0008).*

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### Human Decomposition, Soil Chemistry, Microbial Succession



## B54 Body Mass Index (BMI) Impacts on Soil Chemical and Microbial Responses During Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will understand how body mass index (BMI) influences chemical and microbial patterns in decomposition-impacted soil. This presentation will increase attendees' knowledge of human decomposition processes, including inter-individual variability in decomposition due to BMI, particularly as it relates to the soil environment.

**Impact Statement:** This presentation will impact the forensic science community by furthering the knowledge of cadaver-related factors (intrinsic factors), specifically BMI, and how they impact soil chemical and microbial response during decomposition.

Human decomposition is a complex process that is driven by the activity of decomposer organisms, including soil microbes.<sup>1</sup> Multiple factors can influence decomposer presence and/or activity, including extrinsic (e.g., temperature, moisture, etc.) and intrinsic, cadaver-related factors.<sup>2,3</sup> To date, a majority of decomposition studies have assessed the importance of extrinsic factors in decomposition; however, the effects of intrinsic factors has received little attention.<sup>4</sup> BMI, one intrinsic factor of interest, is the relationship between an individual's weight and height and is commonly used to estimate body fat. Body fat varies between individuals due to sex, age, and health status/medical conditions, thus, BMI of individuals in death investigations is expected to vary.<sup>5</sup> The goal of this work was to assess the effect(s) of BMI on soil chemical and microbial patterns during human decomposition.

Decomposition of 19 deceased human donors was observed through the end of active decomposition (here defined by cessation of decomposition fluid purging from the abdomen). This study took place at the University of Tennessee Anthropology Research Facility between February 2019 – March 2020. BMI was calculated [weight(kg)/height(m)<sup>2</sup>] from reported values and individuals were sorted into BMI categories following CDC BMI levels (Underweight < 18.5, normal 18.5 – 24.9, overweight 25 – 29.9, obese > 30). Control and decomposition soil samples were taken at predetermined accumulated degree hours (ADH) intervals until the end of active decomposition. To understand soil chemical patterns, soil pH and electrical conductivity (EC) were measured. Soil microbial activity was assessed via heterotrophic respiration and extracellular enzyme assays. Co-extracted bacterial and fungal DNA was quantified, and community composition was examined using 16S rRNA gene and internal transcribed spacer (ITS) amplicon sequencing, respectively.<sup>6-8</sup>

Results indicate that an individual's BMI impacts both soil chemical and microbial responses during human decomposition. First, soil pH response is related to donor BMI. For underweight individuals, soil pH increased during decomposition, while decreasing for normal, overweight, and obese donors ( $F = 9.647$ ;  $p < 0.001$ ). This variability in soil pH impacted microbial community activity, specifically leucine amino peptidase (LAP) activity, whereby LAP activity increased with increasing soil pH ( $F = 4.781$ ;  $p = 0.032$ ). While LAP activity was related to soil pH, no relationship was found with donor BMI ( $F = 2.444$ ;  $p = 0.131$ ), suggesting soil pH acts as a control on LAP enzymatic activity. Soil microbial community compositional shifts over time were also impacted by donor BMI. The soil fungal class, Saccharomycetes, was found to increase in decomposition soils of normal, overweight, and obese individuals, but did not increase for underweight individuals ( $F = 10.846$ ;  $p = 0.014$ ).

Taken together, this study provides evidence that donor BMI influences chemical and microbial patterns in decomposition-impacted soil, resulting in inter-individual variability. These results highlight the importance of intrinsic factors in human decomposition and have considerable implications for soil chemical and microbial-based postmortem interval (PMI) models, which rely on consistent responses between individuals.

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### Differential Decomposition, Microbial Ecology, Body Mass Index (BMI)

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**B55 Proteomic Genotyping in Compromised Skeletal Remains**

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**Learning Overview:** This presentation will provide attendees a side-by-side comparison of proteomic- and DNA-based genotyping of degraded forensic anthropological samples.

**Impact Statement:** This presentation demonstrates novel ways by which genotypes can be obtained from degraded skeletons.

This presentation will provide attendees with information regarding a side-by-side comparison of proteomic- and DNA-based genotyping of forensic anthropological samples. This project examines the relative success of DNA and protein levels as well as standard short tandem repeat (STR) typing and GVP typing to identify skeletal remains subjected to a variety of insults including formaldehyde damage, burial, burning, and decomposition.

Skeletal material is often the only remaining evidence in missing persons cases, mass disasters, or mass burials. Nuclear DNA can be highly degraded in these samples making it difficult to recover quality profiles. This is especially true in the highly challenged remains from Ground Zero or the Punchbowl case. Conversely, proteins are chemically more stable than DNA and contain GVPs that can be used to infer a forensically useful autosomal SNP genotype and random match probabilities. Because human remains often undergo multiple stresses, it is important to determine if GVPs can be used to individualize bone samples under a variety of conditions. It is also necessary to examine and compare the quality of results with DNA and standard STRs.

In this study bone samples were subjected to five treatments: embalming, burning, burial and surface decomposition without and with extensive formalin treatment for 0, 0.25, 14, 24, and 66 hours. DNA extraction was performed using PrepFiler BTA chemistry followed by quantification via real time PCR using the Quantiplex Pro Kit and genotyping using the QIAGEN Investigator 24plex Kit. Formalin treatment decreased the overall DNA quantity by 98% percent ( $p = 0.017$ ). Recovery of STRs was more stable with a reduction in alleles detected of only 23%, 26%, 25% and 64% with the increasing time points of formalin treatment. STR results showed a decrease of  $82 \pm 2$  % alleles recovered to  $30 \pm 0$ % after 66 hours. As expected, with the other challenging samples, DNA concentration ranged from 0.0055 ng/ $\mu$ L ng to 9.5539 ng/ $\mu$ L, and STR results ranged from 31% to 100%. After extraction of bone protein after demineralization and protease digestion and mass spectrometry a similar trend was seen when examining total peptide and unique peptide recovery. Total peptide recovery was consistent between the 24- and 66-hour mark and the unique peptides recovery only decreased slightly. Additionally, the DNA input for buried samples was lowest whereas the total peptide amount was highest for these samples. This suggests that with highly degraded samples, proteins are better preserved and can be more readily detected than STRs. There was also larger variation seen between DNA input and the percentage of STR alleles correctly called versus total peptide input and unique peptides called. This variation suggests that using GVPs for identification purposes can provide a more consistent result.

The preservation of peptide information in highly compromised skeletal material led to the hypothesis that the information from genetically variant peptides would also preserve well. GVPs were detected in all samples, the first example of proteomic genotyping in forensic anthropological samples. Depending on the individual 2 to 9 GVPs were detected in skeletonized material. Also, GVP-interpreted SNPs resulted in accurate allele calls. This suggests that in evidence where nuclear DNA is highly degraded protein-based human identification can be used to help determine a genetic profile from multiple sources. More GVPs identifications will occur as refinement and improvements are made in extraction methods of protein from skeletonized material. Proteomic information can then combine with nucleotide-based information to create complex genotypes with higher levels of discriminatory power and utility to forensic investigators.

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**Proteomic Genotyping, Human Identification, Single Nucleotide Polymorphisms**

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## B56 The Use of Proteomic Genotyping to Estimate Genetic Ancestry for Intelligence Purposes

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential of protein-based genetic information to estimate ancestry.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that protein can provide useful genetic information and investigative leads when DNA is compromised or provides ambiguous findings.

Forensic science often requires the generation of leads from trace evidence left at a crime scene. Hair shafts have been used historically for identification and ancestry determination, but these results have become controversial since they depend on pattern-based microscopic comparisons to determine identity and ancestry. Hair shafts do however contain genetically variant peptides (GVPs) with single amino acid changes, the result of non-synonymous single nucleotide polymorphisms (SNPs). Proteomic detection of genetically variant peptides (GVPs) therefore allows the inference of corresponding SNP alleles. Combinations of these particular genotypes can then be used to estimate random match probabilities and relative likelihood of an individual's genetic ancestry.

In this study, proteomic genotyping was conducted on human hair shafts to assess the ability of several GVPs found in order to classify individuals by biogeographic background. Matching hair and saliva were collected from a cohort of 174 individuals for proteomic and genomic genotyping. This cohort consisted of African, European, Asian, Middle Eastern, and admixed individuals. The study identified potential ancestry informative peptide biomarkers by assessing  $F_{ST}$  and  $H_o$  from a list of total observed GVPs ( $n=408$ ), and 11 more when also incorporating sensitivity, linkage disequilibrium, and Hardy-Weinberg equilibrium. In addition, variable importance scores from partial least squares discriminant analysis identified over 50 more GVPs that contribute more heavily to the separation of data. Using a list of 408 validated GVPs, European, African, and admixed individuals were classified by three statistical methods: 1) multivariate analysis of GVP profile, 2) modeling using common population genetics software, and 3) calculation of likelihood ratios using estimates of random match probability.

Population genetics software programs such as STRUCTURE and ADMIXTURE resulted in a variance of 8% between the estimated values of ancestry using 53 discovered GVP SNPs versus 495 known ancestry informative markers using DNA-based SNP genotyping as a proof of concept. Likelihood ratios showed distinct distributions for each population, with a correlation between ratio and per cent African ancestry. Population estimates using proteomically-derived LR values were significantly correlated with genomically-derived per cent African ancestry ( $p<0.01$ ). Improvements in discovery and detection sensitivity will further increase the resolution and statistical power of ancestry inference using GVP generated SNP information. These results indicate that methods of biogeographic classification from proteomic genotyping offer a valid alternative to current methods using hair shafts, particularly as proteomic genotyping becomes more a refined, sensitive and validated method. In sum, proteomics has the potential to provide actionable ancestry intelligence information about a deposited hair sample, regardless of the absence of the hair bulb structure.

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### Proteomic Genotyping, Ancestry Informative Markers, Genetically Variant Peptides

## B57 The Development and Evaluation of Two DNA Collection/Extraction Techniques for Cartridge Cases

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of how two different collection/extraction methods may be used to recover DNA from fired and unfired cartridge cases.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of two DNA collection/extraction methods for the recovery of DNA from fired and unfired brass and nickel-plated cartridges.

For more than 10 years, studies have modified DNA collection, extraction, purification, and amplification with varying levels of success attempting to increase the recovery of DNA from ammunition; two studies were most notable. Montpetit & O'Donnell modified the method developed by Dieltjes et al. by adding DNA concentration following extraction, referred to as the "soaking" method.<sup>1,2</sup> The second method, referred to as "rinse-and-swab" was developed by Bille et al.<sup>3</sup> This method employs two different swab types and an additive solution to reduce the effects of copper degradation on DNA.

The purpose of this project was to compare these two promising DNA collection techniques for fired and unfired cartridge cases. First, cartridges from various manufacturers were tested for the presence of exogenous DNA. Then the two DNA collection methods were evaluated on an inert substrate that does not have metal ion interactions. Next, both methods were tested on brass and nickel-plated cartridges, both fired and unfired. Finally, the two methods were modified in an attempt to increase DNA yield and recovery.

For the exogenous DNA experiment, 210 cartridges derived from nine manufacturers were swabbed and processed using the laboratory SOP, which includes DNA extraction with the EZ1 Advanced XL, DNA concentration, and DNA quantification using Quantifiler Trio. Extracts for which the small autosomal target was quantified (44 of 210) were amplified and genotyped using the GlobalFiler PCR Amplification kit and 3500xL Genetic Analyzer. Of these, only one extract yielded two alleles concluding that the manufacturing process of ammunition is relatively DNA free.

Next, to identify a suitable amount of DNA to spot on cartridges and to obtain a baseline comparison of the two methods, 120 polypropylene 1.1 mL mini tubes were spotted with five concentrations (20ng, 10ng, 5ng, 2ng, and 1ng) of human adult epidermal keratinocytes (HEKa). Twelve mini tubes spotted with each DNA concentration were processed with both the soaking and rinse-and-swab methods. A final concentration of 5ng was chosen for DNA spotting of cartridges for the last two experiments.

Next, both collection methods were compared using brass and nickel-plated cartridges. Briefly, 600 cartridges (300 brass and 300 nickel-plated) were spotted with 5ng of HEKa cells and half were processed using the soaking method and half with the rinse-and-swab method. Each collection method processed 75 brass and 75 nickel-plated cartridges, both fired and unfired. The results show that the soaking method recovered an average of 0.25 ng of DNA or 5.1% recovery, and the rinse-and-swab method recovered an average of 0.28 ng of DNA or 5.8% recovery. Additionally, the results suggest that there is no significant difference in yield between the soaking and rinse-and-swab methods. However, significant differences were observed in fired vs unfired cartridges and brass vs nickel-plated cartridges.

Finally, seven experiments were performed using 25 fired brass cartridges each (n=175) to determine if a hybrid method could be used to increase DNA yield and recovery. These experiments included buffer, incubation, and extraction modifications to both the soaking and rinse-and-swab methods. However, the modifications presented did not result in increased DNA yield or recovery. It was determined that the soaking and the rinse-and-swab methods provided the highest DNA yield and recovery of all modifications tested, and the two methods, once again, did not produce significantly different results.

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### Cartridges, Soaking, Rinse-and-Swab

## **B58 A Comparison of the Effect of Manual Versus Robotic DNA Extraction Methods on the Microbial Communities Associated With Forensically Relevant Human Biological Samples**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the microbial DNA associated with various human biological samples and how manual and robotic extractions (common in crime laboratories) impact the bacterial structure associated with these samples. Attendees will also have a better understanding of the microbial taxa commonly associated with common DNA extraction reagents.

**Impact Statement:** This study will aid the forensic community in establishing more robust extraction protocols and methods, specifically regarding robotic extractions that may not be suitable for microbial evidence.

With the emergence of high-throughput sequencing, culture-independent microbiome analyses are being investigated for forensic applications. However, the forensic science biological sample workflow was not designed with microbial evidence in mind, so additional precautions must be clearly defined and implemented. Common forensic DNA extraction kits are not certified to be bacteria free, and it is therefore critical to identify how reagent-based microbes impact the microbial signatures associated with forensically relevant body fluids.<sup>1,2</sup> This will not only define the reagent-based impacts on our previously developed microbiome-based body fluid identification method but will also define the impact of robotic extraction on these signatures.

This study was designed to investigate how the extraction methods influence the microbial signatures in body fluids. Saliva, feces, vaginal fluid, and menstrual blood were collected in duplicate using an IRB approved collection method. Ten samples of each body fluid were extracted using the standard Forensic Casework Sample protocol on the QIAcube, while the duplicate samples were extracted manually using the QIAamp DNA Investigator Kit according to manufacturer's protocol. All body fluid samples were extracted alongside body fluid specific reagent blanks to evaluate the differences between blank samples extracted robotically versus those extracted manually. This was to determine whether extraction methods impact the presence, absence, and abundance of bacterial taxa, as well as determine whether extraction methods impact the likelihood of cross contamination in reagent blanks.

Immediately after extraction, the V4 region of 16S rDNA was sequenced on MiSeq FGx sequencing platform following the dual-indexing protocol as described by Kozich et al.<sup>3</sup> Sequences were then analyzed using mothur version 1.45.3, and statistical analysis was performed using R version 3.6.1.<sup>4,5</sup>

With the exception of one robotically extracted reagent blank associated with fecal samples, all reagent blanks had DNA concentrations below the limit of detection. A preliminary Analysis of Molecular Variance<sup>6</sup> comparing reagent blanks only, indicated that there was a significant difference between the reagent blanks extracted manually versus those extracted robotically.<sup>6</sup>

Findings from this study will help minimize errors associated with the accuracy of microbial signature-based method for body fluid identification, while enhancing current forensic protocols.

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### **Body Fluid ID, Microbiome, Robotic Extraction**

## B59 Using Bone Biology to Optimize Forensic and Ancient DNA Extraction From Post-Cranial Skeletal Elements

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**Learning Overview:** The overarching hypothesis of this project is that endogenous DNA levels in post-mortem human bone are primarily dependent on cellular and biochemical factors. More specifically, extraction sites on, or adjacent to, regions of *in vivo* bone remodeling will yield higher amounts of, and better quality, nuclear and mtDNA than extraction sites where cellular populations are minimal. Additionally, biochemical factors, such as increased crystallinity, will be reflected in extracted DNA quality and quantity and can be predicted using ATR-FTIR technology. The project also inspires an array of further research involving testing these methods, hypotheses, and theoretical underpinnings across multiple individuals and varying taphonomic conditions. This framework can also be used to test and track changes in cellular populations in different ages at death, as well as investigating the cellular and biochemical foundations of the various skeletal pathologies.

**Impact Statement:** Beyond expanding our knowledge, in many forensic and archaeological recoveries, or instances of remains surrendered to an ME/Coroner, the petrous and/or teeth are not present. Therefore, pinpointing the ideal sites for DNA extraction will reduce cost, improve efficiency, and aid in avoiding ethical dilemmas pertaining to destructive analysis of human remains. Research projects and paradigms across anthropology will benefit from an enhanced understanding of the reasons behind differential DNA preservation. This project also allows for an array of teaching opportunities. Undergraduates in both Anthropology and Chemistry Departments have already begun learning about the ethical treatment of human remains, proper drilling techniques, sampling protocols, aDNA and forensic DNA extraction and analysis, and experimental design.

The optimal site for DNA extraction from human skeletal remains lies primarily in the petrous portion of the temporal bone, and secondarily in the dental pulp, though the reasons for this primacy remain a mystery. Studies involving post-cranial remains, however, rely heavily on prior publications or experience, and less on foundational knowledge of cellular populations or biochemical processes and success rates vary widely. In fact, no current studies of skeletal remains exist that incorporate deep understanding of the cellular and biochemical components of bone as a potential reason for differential DNA preservation between or within post-cranial elements. Most of the current literature focuses on the primacy of taphonomic factors in differential DNA preservation. Many such studies do acknowledge and lament the lack of foundational understanding of the cellular and biochemical contribution to forensic and ancient DNA.

This research seeks to fill that void. In this project, over 400 extraction sites, representing cortical, trabecular, and lamellar bone tissue types, were sampled from across the post-cranial skeleton from a single individual, as were the petrous and the right, maxillary 1st molar (RM1). Since it is known that bone cell populations differ across the skeleton, these extraction sites were carefully selected for either their roles in skeletal homeostasis, visible trauma or pathology, or previously documented failure to routinely yield enough DNA for a full STR profile. Scanning electron microscopy (SEM) helped visualize areas of osteoclastic activity and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) technology biochemically corroborated the presence of endogenous DNA in each sample prior to further destructive analysis. Each site will be run for both mitochondrial DNA (mtDNA) and the 21 CODIS STR markers. Oneway ANOVA testing will help clarify variance found in bone tissue types, skeletal element, and sites per element across the post-cranium as a whole and Chi-square analysis of independence may help clarify inter-element differences in endogenous DNA yields. Goodness of fit testing will compare post-cranial yields to the dental and petrous samples.

Results of ATR-FTIR analysis shows the presence of endogenous DNA in all 400 samples taken from the post-cranium as well as in petrous and dental samples. These results were also compared to ancient samples that had failed to yield endogenous DNA in previous studies. However, no statistical difference was found in biochemical component analysis between cortical and trabecular bone samples.

SEM imaging successfully indicated the presence of Howship's lacunae on both the auricular surface and the pubic symphysis, confirming the ante-mortem presence of multinucleated osteoclasts in the process of homeostatic bone resorption.

The use of a single individual (recovered from cold, fresh, water and professionally macerated at the Montana State Crime Lab in Missoula, MT, USA) for this study is ideal because it controls for many hidden and often confounding variables introduced by taphonomic and diagenetic processes. Such control allows for a purer analysis of the cellular and biochemical contributions to endogenous DNA extraction. Ethical concerns over destructive analyses involving human remains are also eliminated, as the individual (identified through CODIS by soft-tissue DNA extraction prior to maceration) had no familial obligations.

Uniquely, this project: (1) utilizes knowledge of the cellular components and biochemical processes specific to the growth and maintenance of bones to target specific sites on skeletal elements for optimal DNA extraction, (2) incorporates knowledge of cell types to investigate the specific type of bone (cortical or trabecular) that is best for sampling at that site, (3) will design and construct a visual "heat map" of the human post-cranial skeleton for use in both forensic DNA and ancient DNA (aDNA) laboratories.

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### Anthropology, DNA, Osteology

## B60 Compositional Analysis of Human Skeletal Samples Using Raman Spectroscopy and Correlation to DNA Recovery

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the complexities associated with DNA extraction from bone, and how Raman spectroscopy may be used to understand the correlation between bone microstructure and DNA preservation.

**Impact Statement:** This presentation will impact the forensic science community by initiating an investigation into the use of Raman spectroscopy as a non-destructive screening tool for predicting the potential for DNA recovery from bone.

The purpose of this study is to determine if Raman spectroscopy can be used as a rapid and non-destructive screening tool to predict DNA recovery potential for bone, and to explore the correlation between the compositional structure of bone and preservation of DNA. The two primary structural components of bone are calcium hydroxyapatite and collagen.<sup>1,3,4,6-11</sup> It is proposed that by analyzing bone using Raman spectroscopy, the presence, state, and distribution of hydroxyapatite and collagen can be measured, which may be useful in predicting if the sample is viable for genetic testing.

Currently, a reliable screening method for DNA extraction from bones does not exist. Previous studies demonstrate that certain skeletal elements (e.g., weight-bearing long bones, molar teeth) are preferred for forensic DNA testing; however, once these skeletal elements are selected, the sampling technique is blind.<sup>1,2,6,9</sup> Moreover, the external physical appearance of bone has been proven to not be a reliable predictor of DNA recovery potential.<sup>2</sup> During postmortem diagenesis, the microstructure of bone decomposes in a non-uniform manner. The heterogeneous nature of bone diagenesis poses a challenge in determining which region(s) of the bone contain the most intact microstructure (and presumably the most DNA).<sup>5</sup> The diaphysis of a long bone is the optimal area for sampling because it is comprised of very dense compact bone, which provides a strong physical barrier to environmental insult, and contains the highest concentration of osteocytes (nucleated bone cells that possess DNA).<sup>9</sup> With Raman spectroscopy, it is proposed that the entire diaphysis can be sequentially scanned, and data collected from each scanned region can provide qualitative and quantitative information regarding bone microstructure (i.e., calcium hydroxyapatite, collagen), which may correlate to the quality and quantity of DNA present in each region. Forensic DNA analysis of human remains is time-consuming, labor-intensive, and expensive. This pre-screening tool has the potential to provide analysts with preliminary data to make informed decisions on the location of optimal sampling sites along the diaphysis of a long bone, thereby reducing the amount of bone consumed and destroyed in the testing process. Raman spectroscopy could offer a robust and reliable method to expedite and streamline the processing of unidentified skeletal remains in casework.

Analysis of results will be conducted to assess the relationship between the decomposition state of bone microstructure and DNA recovery, and to determine the reliability of Raman spectroscopy as a screening method. Preliminary research suggests that higher quantities of DNA (and better quality DNA) can be recovered from bone samples containing preserved hydroxyapatite and collagen versus samples taken from regions of the same bone that are in a more advanced state of diagenesis. DNA quantification using the Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio 5 Real-Time PCR System will provide data on total DNA recovery from each scanned bone section, as well as a quality index to indicate the degree of DNA degradation present.

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### Raman Spectroscopy, Bone Diagenesis, DNA Preservation

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## **B61 An Enhanced DNA Extraction Method for Charred Bone Remains Using Pressure Cycling Technology (PCT)**

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**Learning Overview:** After attending this presentation, attendees will understand a pressure cycling technology (PCT) enhanced DNA extraction method for working with charred bone remains.

**Impact Statement:** This presentation will impact the forensic science community by presenting a DNA extraction method that could yield a higher quantity and quality of results from charred bone remains.

Charred human skeletal samples may be submitted for forensic DNA testing from a variety of scenarios, including arson, wildfires, vehicular accidents, accidental fires, and terrorist attacks. Extraction of DNA from charred bones is particularly challenging. Exposure to heat damages DNA, thereby reducing the quality and quantity of genetic data that can be recovered to assist in making a positive identification. Bone itself presents a complicated matrix to work with for extractions, as minerals present within the bone microstructure can act as PCR inhibitors and create downstream issues in the analysis of the DNA.

In this study, the application of Pressure Cycling Technology (PCT) was used in an attempt to enhance the extraction of DNA from charred bone remains and increase yield. Metacarpal bones were burned at various, pre-defined incremental temperatures in a muffle furnace. After burning, half of the bones were subjected to standard DNA extraction protocols, while an additional PCT step was incorporated into the protocol for the other set of samples. DNA yield, as well as the quality of the profiles generated, were compared between the two experimental groups. A modified organic extraction was utilized for both sets of bones, with the additional PCT step occurring during lysis for the experimental group. Extracted DNA was quantified using the Quantifiler™ HP DNA Quantification Kit (ThermoFisher Scientific) and an Applied Biosystems QuantStudio 5 Real-Time PCR System. The samples suitable for typing were amplified using the GlobalFiler™ PCR Amplification Kit and a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems). Amplified product was genotyped using an Applied Biosystems SeqStudio Genetic Analyzer and GeneMarker® HID Human Identity Software.

Pressure cycling has been shown to reduce the effect of inhibitors in the PCR process, generating cleaner profiles and higher yields from extracted samples.<sup>1</sup> By exploring and optimizing methods to extract DNA from charred bone remains, genetic recovery from bodies compromised by fire could be improved, thereby assisting in the resolution of a greater number of unidentified human remains (UHR) cases.

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### **Pressure Cycling Technology, DNA Extraction, Charred Bones**



## B62 Alternative Sperm Cell Lysis Methods for Sexual Assault Samples

Sarah K Schellhammer, MS, Virginia Commonwealth University, Richmond, VA; Brittabny C. Hudson, MS\*, Virginia Commonwealth University, Richmond, VA; Jordan O. Cox, MS, Virginia Commonwealth University, Richmond, VA; Tracey Dawson Green, PhD, Virginia Commonwealth University, Richmond, VA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of alternative sperm lysis techniques that reduce the time and costs associated with processing sexual assault samples, as well as how those methods compare to traditional approaches.

**Impact Statement:** This presentation will impact the forensic science community by presenting data which demonstrate the availability of several alternative sperm cell lysis techniques that reduce time and cost of processing while providing high quality STR profiling results; thus, it will offer a tool to help combat the current backlog.

The prevalence of sexual assault kit backlogs in the United States has spurred research aimed at providing more efficient ways for handling associated samples. Traditional forensic DNA workflows for sexual assault samples include cell separation, cell lysis, and DNA purification, all of which are time and labor intensive, hindering the ability of forensic analysts to keep pace with evidence submissions.<sup>1-5</sup> These issues have prompted the recent exploration of new techniques for each of these steps, as well as new workflows that omit purification altogether. Thus, this study evaluated seven alternative techniques for cell lysis with the goal of developing a method for sexual assault samples that could allow for the omission of lengthy purification and transfer steps, efficiently lyse spermatozoa, consistently generate high quality STR profiles, and reduce reagent consumption, time, and associated costs.

Semen from ten donors was dried onto foam swabs, and 1/12<sup>th</sup> swab cuttings were tested in triplicate using commercial (e.g., SwabSolution™, Casework Direct, forensicGEM™ Sperm), “homebrew” (e.g., NP-40 buffer and alkaline lysis solution), and natural sperm decondensation (e.g., HEPES/Triton X-100 [HTX] and HTF media/glutathione/heparin [HGH]) approaches. Several volumes, reagent concentrations, and incubation times were evaluated with the goal of minimizing costs and sample loss/consumption. Samples lysed using forensicGEM™ Sperm served as the control group for all comparisons and statistical analyses. Quantification of human DNA yield using Investigator® Quantiplex HYres revealed that all lysis methods performed comparably to the control method of forensicGEM™ Sperm ( $p > 0.06$ ). All internal PCR controls (IPCs) crossed the cycle threshold within the acceptable range; however, IPCs associated with all SwabSolution™ and some Casework Direct lysates were slightly delayed.

Following DNA quantification, lysates were amplified via the Promega™ PowerPlex® Fusion 5C System using two approaches: 1) a targeted DNA input (ng), based on quantification results and an internally validated protocol, and 2) a targeted volume input (μL) for situations in which quantification may not be performed ahead of “direct” amplification. Amplicons were separated using the ABI® 3130 Genetic Analyzer. Amplification of lysates with a target DNA input (0.25 ng) revealed samples that were lysed with Casework Direct, alkaline lysis, and NP-40 lysis buffer techniques produced DNA profiles with average STR peak heights comparable to the control method, as well as interlocus balance similar to or better than the control ( $CV \leq 0.38$ ). STR amplification using a specified volume of lysate rather than a target DNA input generated similar results, with HGH cell lysis also providing high quality STR profiles.

Overall, this study demonstrated the ability of Casework Direct, alkaline, NP-40, and HGH methods to efficiently lyse spermatozoa and provide high quality STR profiles despite the absence of a purification step. Ultimately, based on the data reported herein, alkaline lysis is the most highly recommended and preferred alternative approach for sperm lysis, as it produces high quality profiles, saves time, and substantially decreases the cost per reaction when compared to traditional lysis methods. Further, this technique provided optimal results when using both a targeted DNA and targeted volume input, demonstrating its potential for other applications such as direct-to-PCR amplification and microfluidic devices.

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### Sexual Assault, Cell Lysis, Direct Amplification

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**B63 An Evaluation of a Body Fluid Identification Method Using MicroRNAs (miRNAs) Co-Extracted With DNA**

*Kelsey Price, BS\*, Virginia Commonwealth University, Richmond, VA; Carolyn Lewis, BS, Virginia Commonwealth University, Richmond, VA; Ciara Rhodes, BS, Virginia Commonwealth University, Richmond, VA; Anaya Valentine, MS, Houston Forensic Science Center, Houston, TX; Edward Boone, PhD, Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, VA; Sarah Seashols-Williams, PhD, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA*

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**Learning Overview:** After this presentation, attendees will understand how microRNA (miRNA) expression can be used for body fluid identification without the need for separate DNA and RNA extractions. The presentation will inform attendees about the limitations of the method, and attendees will understand why this molecular-based method may be a more reliable method for body fluid identification in forensic casework than current enzyme-based serological tests, which are prone to false positives.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the evidence for using miRNAs for body fluid identification over current methods in forensic casework continues to grow.

MiRNAs are non-coding RNAs 18 to 25 nucleotides in length. Due to their smaller size, the absence of a poly-A tail, and protein encapsulation in vivo, miRNAs are less susceptible to degradation when compared to messenger RNAs. Additionally, previous studies have demonstrated that miRNAs co-elute with many DNA extraction methods, thus potentially eliminating the need for an additional RNA extraction and therefore simplifying the implementation of this method into the forensic laboratory workflow.

Several candidate miRNAs were previously identified using high-throughput sequencing (HTS) of the miRNome. The differential expression patterns of miRNAs were analyzed using quantitative PCR (qPCR) in forensically relevant body fluids. Candidate miRNAs and endogenous reference markers were identified using these data and validated with population sample sets of RNA extracts from each body fluid.

In this study, the candidate and endogenous reference miRNAs were evaluated using DNA extracts from dried venous blood, menstrual blood, saliva, vaginal secretions, feces, urine, and semen. The samples were deposited onto 50 swabs per body fluid, allowed to dry, and extracted using a silica-based DNA extraction method. Then, the miRNA levels were measured using reverse-transcription quantitative PCR (RT-qPCR). Next, differential expression of the miRNAs within each body fluids within the population set were analyzed, normalized, and evaluated with a quadratic discriminate analysis model. This model was adapted for miRNA expression from DNA extracts in the sampled biological fluids using this panel and demonstrated a correct classification accuracy rate of 91.36%. Finally, the relative stability of miRNA in dried body fluids under degrading chemical, heat, and environmental conditions meant to mimic a crime scene and over time within an individual was also evaluated in DNA extracts. These samples were classified using the same quadratic discriminate analysis model.

In conclusion, the panel of nine miRNA markers were shown to have a high specificity for identifying all biological fluids tested except for menstrual blood and vaginal fluid using quadratic discriminate analysis in DNA extracts and is a promising alternative to traditional serological tests for forensic casework.

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**Body Fluid Identification, MiRNA, MiRNA Stability**

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### **B64 A Demonstration of a Semi-Automated, Extraction-Free, MicroRNA (miRNA) Gene Expression Profiling Method for the Simultaneous Detection of Biofluids**

*Amy Brodeur, MFS\*, Boston University School of Medicine, Boston, MA; Frank Kero, PhD, HTG Molecular Diagnostics, Tucson, AZ; BJ Kerns, BS, HTG Molecular Diagnostics, Tucson, AZ*

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**Learning Overview:** By attending this presentation, attendees will learn about the potential for microRNA (miRNA) profiling to be used for identifying body fluids present within a forensic stain.

**Impact Statement:** This presentation will impact the forensic science community by offering an alternative to traditional chemical and serological assays for identifying body fluids.

Biological stains recovered from crime scenes may be complex, limited in size and not always visually consistent with the body fluids of which they are comprised. The identification of specific body fluids can provide important contextual information, particularly in cases concerning sexual assault. Further, the presence or absence of certain biological material may help guide DNA analysis or impact its probative value.

Current forensic practice for body fluid identification is to employ colorimetric chemical tests and protein-based testing in the form of lateral flow immunochromatographic test strips. These assays are primarily presumptive in nature and are capable of detecting only one body fluid at a time, so multiple tests and samples are needed for a complete evaluation. A more specific, multiplex approach to forensic body fluid identification would be highly advantageous.

The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) was developed to measure the expression of human miRNA within the HTG EdgeSeq system workflow. The HTG EdgeSeq system combines HTG's proprietary quantitative nuclease protection assay (qNPA) chemistry with a next-generation sequencing (NGS)-based platform to enable the quantitative analysis of a panel of targeted genes in a single assay. The HTG EdgeSeq miRNA WTA contains 2,102 nuclease protection probes (NPPs), including 13 housekeeping genes, one internal positive control, and five internal negative controls.

Human blood, semen, saliva, urine, menstrual blood, and breast milk were collected from various volunteers following an approved IRB protocol. Fifteen microliters of each fluid were added to an equal amount of HTG lysis buffer. Functional DNA nuclease protection probes (NPPs) were hybridized to target miRNAs. S1 nuclease was then added to digest excess non-hybridized DNA probes and non-hybridized miRNA, leaving only NPPs hybridized to miRNA fully intact and able to be barcoded. Lastly, the NPPs were quantified by NGS. Principal component analysis (PCA) was used to assess the global distribution difference across the various miRNAs and specimen types.

Following PCA analysis, a clear separation of the types of biofluids tested was shown. As an example, 187 miRNAs between saliva and aspermatic semen, 92 miRNAs between saliva and semen and 77 miRNAs between aspermatic semen and semen were found to drive this differentiation. Preliminary results suggest that the use of miRNA as a method for differentiating between body fluids is feasible and may be a suitable method for the forensic identification of mixed biofluid stains.

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#### **MiRNA, Body Fluid Analysis, Next Generation Sequencing (NGS)**

## B65 Direct Messenger RNA (mRNA) Sequencing for the Identification and Analysis of Human Body Fluids

*Quentin Gauthier, PhD\**, Federal Bureau of Investigation, Quantico, VA; *Patrick Rydzak, PhD*, Federal Bureau of Investigation, Quantico, VA; *Kyleen Elwick, PhD*, Federal Bureau of Investigation, Quantico, VA; *Mark Kavlick, PhD*, Federal Bureau of Investigation, Quantico, VA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of advancements in nanopore sequencing technologies and how they can be applied to body fluid identification.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a novel method for body fluid identification using a straightforward analytical approach that can be adopted with relative ease in forensic laboratories.

Body fluid identification via analysis of differential expression of messenger RNA (mRNA) is well understood in the scientific community and particularly relevant to the field of forensics.<sup>1</sup> Source attribution of body fluid stains found on submitted evidence affords forensic DNA analysts' insight on which samples would provide the greatest evidentiary value for future processing after co-isolation of DNA and RNA.<sup>2</sup> Much of previous RNA body fluid identification work has commonly employed a reverse transcription (RT) reaction to generate complimentary DNA (cDNA), a step that may introduce bias in RT-qPCR assays and RNA sequencing assays.<sup>3</sup> Recent advancements in RNA sequencing technology have bypassed the RT step and increased the lengths of reads for more streamlined and accurate data analysis.

This study employed Oxford Nanopore Technology's (ONT) direct RNA sequencing technology to identify and analyze mRNA markers within body fluids without a cDNA intermediary. TRIzol was used to extract RNA from saliva, blood, and semen, which was then prepped using Oxford Nanopore Technology's direct RNA sequencing protocol. RNA libraries were prepared from total extracted RNA standards directly, as well as post-polyadenylation, with External RNA Controls Consortium (ERCC) spike-in to act as an internal control. Sequencing was performed using MinION flow cells (R9.4.1) on the MK1C sequencing instrument. Data generated this way was analyzed for the presence of body fluid-specific mRNA markers corresponding to either saliva, blood, or semen, and for the absence of mRNA markers from other sources, such as vaginal fluid, menstrual blood, urine, and touch RNA. Reads from each sample were also queried against and aligned to the human transcriptomic reference GRCh38 using BLAST+ and Minimap2 respectively. Significant matches between sample reads and transcriptomic sequences were categorized and the top matches for each RNA standard were noted.

Of the RNA samples tested, mRNA markers unique to that tissue type were detected in the saliva, blood, and semen samples offering promising preliminary results for the use of direct RNA sequencing for body fluid identification. Ongoing research includes the analysis of total RNA extracted directly from additional tissue types, such as vaginal fluid, menstrual blood, urine and touch RNA, as well as the refinement of the bioinformatics data analysis pipeline to ensure that reads are matched to the correct transcripts with a high level of confidence.

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### Body Fluid Identification, Direct RNA Sequencing, Oxford Nanopore Technology

## B66 The Development of Pyrosequencing-Based DNA Methylation Assay for Menstrual Blood Identification

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**Learning Overview:** After attending this presentation, attendees will be aware of the development of a novel and sensitive DNA methylation assay for menstrual blood identification using pyrosequencing chemistry.

**Impact Statement:** This presentation will impact the forensic science community by providing the analysts with a scientific tool to differentiate menstrual blood from peripheral blood as well as vaginal epithelial cells closer to menstruation.

In forensic casework, DNA is extracted from biological exhibits and compared to databases. Yet, in cases where an offender has regular access to a victim, a DNA profile may not be probative unless the source of the biological stain is known. For instance, a caregiver may claim that DNA found on their clothing results from an innocent transfer. However, if this DNA originates from vaginal epithelial cells, a crime may have occurred. Body fluid analysis thus can be critical in prosecuting violent crimes such as rape or child abuse.<sup>1</sup>

Current tests used to identify body fluids are limited in specificity particularly when compared with DNA detection methods such as the PCR. Therefore, the forensic community is developing more advanced techniques, such as epigenetic methylation assays, to distinguish body fluids: blood, saliva, semen, and vaginal epithelia.<sup>2-3</sup> Markers for menstrual blood, are challenging due to heterogeneity: blood, endometrial lining tissue, vaginal and cervical secretions may all be present. Thus, primers were designed for methylation markers within the SLC26A10 and MDFI genes.<sup>4-6</sup> Menstrual blood was collected on cotton swabs daily from female volunteers, DNA was extracted, bisulfite converted, amplified, and pyrosequenced. Methylation data was then interpreted using hierarchical cluster analysis. Markers on the SLC26A10 showed significant methylation percent differences compared to peripheral blood whereas markers on MDFI showed hypermethylation mainly on days 2-3, suggesting a greater presence of endometrial lining tissue.

In this study we have examined swabs from a set of female volunteers to track changes in DNA methylation across the menstrual cycle. Our results demonstrate variations in methylation levels which are consistent with time-based variation in shed cells and further demonstrate the applicability of multiple epigenetic loci in the detection of menstrual blood.

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### Menstrual Blood, Pyrosequencing, DNA Methylation

## B67 The Optimization of a Rotationally Driven Microfluidic Method for Forensic DNA Methylation Sample Preparation

Rachelle Turiello, MS\*, University of Virginia, Charlottesville, VA; Leah Dignan, BS, University of Virginia, Charlottesville, VA; Francine Garrett-Bakelman, MD, PhD, University of Virginia, Charlottesville, VA; James Landers, PhD, University of Virginia, Charlottesville, VA

**Learning Overview:** After attending this presentation, attendees will have been introduced to a rotationally-driven microfluidic method for sodium bisulfite conversion of differentially-methylated DNA in preparation for forensic epigenetic analysis.

**Impact Statement:** This presentation will impact the forensic science community by contributing a robust analytical method for epigenetic sample preparation that is amenable to forensically-relevant amounts of input DNA.

In recent years, forensic genetics analysis has transcended comparative methods for human identification, such as short tandem repeat (STR) testing, to include DNA phenotyping.<sup>1</sup> In particular, the development of a molecular method for epigenetic age determination has garnered considerable attention, as it would not only contribute to the creation of a biological profile, but also inform other phenotypic traits including hair color and loss, stature, and general physical appearance.<sup>2</sup> However, these methods invariably require extensive sample preparation to extract, purify, and subsequently modify the DNA sequence for downstream analysis. The latter process, known as Bisulfite Conversion (BSC), preferentially converts unmethylated cytosines to uracil, thereby distinguishing age-associated methylation patterns within the genome for further genetic and statistical analysis.<sup>3</sup> Unfortunately, current BSC techniques are known to result in extensive DNA fragmentation and loss, and are time-consuming, labor-intensive, and pose high risk for human contamination due to numerous open-tube pipetting steps.<sup>4,5</sup>

The goal of this work is to design and optimize a robust microfluidic platform for silica superparamagnetic bead-facilitated BSC to enable rapid, efficient conversion of forensically-relevant DNA input masses in an automated, enclosed, and rotationally-driven microCD ( $\mu$ CD) format. Leveraging microfluidics to facilitate BSC for forensic laboratories is ideal for several reasons. First, fluid volumes may be reduced significantly, decreasing the amount of input DNA required, reagent costs associated with sample preparation, and required incubation times. Second, the cost-effective fabrication of the enclosed  $\mu$ CDs make them well-suited for implementation into the forensic genetics regime, as they may be discarded as consumables and are less susceptible to analyst contamination. Third, and finally, the integrated, on-board microfeatures ablated into the polymeric device accommodate all BSC-associated chemistries and sequential unit operations to permit comprehensive, automated BSC with no required user intervention. Ultimately, each  $\mu$ CD is capable of simultaneous BSC of four samples, each with a minimum requirement of 2  $\mu$ L isolated input DNA. Reagent fluid volumes are also reduced to approximately 10% of the volume used in the traditional format, along with a decreased total analytical time.

Microfluidic architecture designed using AutoCAD software was tested for fluidic handling by pairing aqueous dye studies with objective image analysis using image analysis software. Early optimization of BSC chemistry was completed in-tube at both full-scale and using decreased fluid volumes to mimic volumes used in the microfluidic regime. BSC of both 0% and 100% methylated control DNA was assessed via real-time polymerase chain reaction (PCR) with an intercalating dye. In-tube BSC chemistry was then adapted to the  $\mu$ CD, and optimized to decrease on-board heated incubation times, characterize BSC recovery, and confirm sample-to-sample reproducibility using both real-time PCR and pyrosequencing. To ensure forensic relevance of our BSC  $\mu$ CD, all genetic regions interrogated during assay validation were extrapolated from human epigenetic aging literature and sample types, including process controls and extracted DNA from human K-562 erythroleukemia cell lines.

Continued work with this technology will focus on integration of on-board direct-from-swab DNA extraction upstream of BSC to comprehensively address sample preparation. Ultimately, development of a mechatronic platform for  $\mu$ CD automation will limit manual user intervention for swab placement and initiating the sample preparation protocol via the corresponding mechatronic system to perform via a graphical user interface (GUI).

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### Forensic Epigenetics, Sodium Bisulfite Conversion, Centrifugal Microfluidics

### **B68 A Quantiplex™-High Resolution Melt (HRM) Integrated Assay for Early Mixture Detection Using Support Vector Machine Modeling for “Whole Curve” Analysis/Proof-of-Concept Testing**

*Chastyn Smith, BS\*, Virginia Commonwealth University, Richmond, VA; Andrea Williams, MS, Bode Technology, Lorton, VA; Hannah Wines, MS, Houston Forensic Science Center, Houston, TX; Darianne Cloudy, MS, American Type Culture Collection, Manassas, VA; Jordan Cox, MS, Virginia Commonwealth University, Richmond, VA; Dayanara Torres, BS, Virginia Commonwealth University, Richmond, VA; Sarah Williams, PhD, Virginia Commonwealth University, Richmond, VA; Edward Boone, PhD, Virginia Commonwealth University, Richmond, VA; Tracey Dawson Green, PhD, Virginia Commonwealth University, Richmond, VA*

**Learning Overview:** After attending this presentation, attendees will be more aware of a new tool that allows for mixtures to be discerned earlier in the forensic DNA workflow.

**Impact Statement:** This research will impact the forensic science community by providing a qPCR-based quantitation method that produces additional information about an evidentiary sample, which could help shape or redirect the path of an investigation prior to the development of an STR profile.

Analysis of evidentiary samples containing DNA from multiple contributors (“mixtures”) is a time intensive process for a forensic analyst and one where the contributor nature of a sample is not revealed until the end of the traditional forensic workflow. Often, at this stage, retesting or additional testing of mixture samples may not be possible, particularly if only trace amounts of a contributor’s DNA is present. Thus, a new method that would allow for the quick and accurate, identification of single source (versus mixture) samples prior to the end-point of STR analysis would be beneficial. To meet this need, an HRM screening assay has been developed and integrated into an existing step in the laboratory evidence workflow - the real-time PCR-based DNA quantification step. Using the developed assay, resulting HRM data is coupled with prediction modeling approaches to allow for the contributor status of an evidence item to be identified and a genetic comparison to be made without additional steps or delays in processing. The developed integrated quantification-HRM mixture screening assay adds two new amplification targets (STR loci D5S818 and D18S51) and an intercalating dye to an existing commercial human DNA quantification chemistry, the Investigator Quantiplex® kit, which is used in combination with increased denaturation and annealing/extension times along with added transition and melt steps post-amplification. Initially, a subset of the resulting HRM data and a linear discriminant analysis (LDA) were used to predict whether a sample was a single source or a mixture, and if single source, the genotypes of each STR locus. Data was previously presented demonstrating that the modifications to the assay did not impede the accuracy of the quantification reaction nor alter the subsequent melt curves produced. However, accurate predictions of single source sample genotypes were lower than desired. Thus, alternate statistical approaches that incorporated the entire melt curve dataset, rather than key characteristic points, were subsequently investigated.

In the current study, 101 single source samples and 20 two-person mixture samples were tested using the Quantiplex®-HRM integrated assay and the entire HRM datasets were exported for prediction modeling using both LDA and support vector machine (SVM) algorithms in R Studio software. SVM was selected for evaluation as it has the ability to more accurately classify large datasets. For proof-of-concept, only 7 different genotypes were represented (for each locus) in testing. A portion of the samples tested was used to “train” the software and the remaining sample data was used as unknowns (or “validation”) samples for prediction. All samples were tested twice – once on the Rotor-Gene® Q platform and then on a more modern qPCR platform, the QuantStudio™ 6. When tested on the Rotor-Gene® Q the assay exhibited an overall accuracy of 89.39%, correctly classifying 87.5% of single source samples and 100% of mixture samples as such. When tested on the QuantStudio™ 6, the assay performed similarly, exhibiting an overall accuracy of 87.88%, correctly classifying 87.5% of single source samples and 90% of mixture samples. However, genotyping accuracy of the identified single source samples was lower than expected at 60.4% for D5 and 46.2% for D18 on the Rotor-Gene® Q and 16.0% for D5 and 22.6% for D18 on the QuantStudio™ 6. Thus, additional prediction modeling tools would need to be evaluated if one desires to use the resulting single source genotyping data for reliable early exclusionary information.

Overall, this work has successfully confirmed that the Quantiplex®-HRM qPCR-based integrated assay can be used for accurate prescreening identification of mixtures across two different qPCR platforms. Future work will seek to identify the limitations of this assay and to incorporate this HRM assay into more commonly used commercial quantification kits.

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#### **High Resolution Melt (HRM), Mixture Screening, Prediction Modeling**

## **B69 The Integration of a High Resolution Melt (HRM) Mixture-Prescreening Assay Into the Quantifiler® Trio Quantification Chemistry Using the QuantStudio™ 6 Flex Real-Time Polymerase Chain Reaction (PCR) System**

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**Learning Overview;** After attending this presentation, attendees will be informed of ongoing efforts to integrate a previously developed high-resolution melt (HRM) curve assay into the Quantifiler Trio™ kit. This assay, partnered with statistical analysis and prediction modeling, can provide crucial sample information and potentially exclusionary information earlier in the forensic DNA workflow.

**Impact Statement:** This presentation will impact the forensic community by providing a methodology that can detect and distinguish mixtures and single-source evidentiary DNA samples using a more widely adapted qPCR kit and real-time PCR platform. Currently, the forensic DNA workflow is not capable of providing conclusory information about evidentiary samples prior to end-point analysis. This shortcoming can exacerbate the challenges inherent in mixtures and low-template DNA samples. Provided additional sample information earlier in the workflow, protocols could be implemented to mitigate these challenges. To this end, a proof-of-concept HRM assay of D5S818 and D18S51 STR loci using EvaGreen® intercalating dye was integrated into the Investigator Quantiplex® qPCR kit. When tested on the QuantStudio™ 6 qPCR platform, resulting melt curve datasets and sequential statistical analysis were capable of distinguishing single-source samples from mixtures at an 87.88% rate.<sup>1,2</sup>

Given this initial success, integration of the HRM assay into a more commonly used chemistry, the Quantifiler Trio™ kit, was pursued. Unfortunately, the presence of EvaGreen® dye caused an 11.7% increase in the small autosomal (SA) and 35.8% increase in Y quantification values in both female and male samples, rendering this data unreliable. This is likely due to spectral emission overlap between the EvaGreen® dye and VIC™/FAM™ dyes used in the Quantifiler Trio™ assay. Additionally, melt products produced by the kit targets overlapped with the melt range of the STR products. Given these results, new fluorescent dyes were sought that least overlapped with the kit's quantification targets. However, since the kit utilizes all dye channels on the QuantStudio™ 6, the two candidate dyes (SYTO™ 17 and SYTO™ 64) shared a channel with the IPC. As predicted, both dyes interfered with the IPC, changing its expected Ct values, however, both dyes produced IPC Ct values that were consistent across samples and within an accepted range. Additionally, SYTO™ 64 was seen to inflate the large autosomal (LA) target while SYTO™ 17 was detected in the passive reference dye channel. Since these values are normalized to produce the final concentration values, and the standards used for quantification include the same integrated reaction components as the samples, it was less of a concern.

In order to keep the integrated assay chemistry at concentrations comparable to the Quantifiler Trio™ assay, two reaction conditions, at 11µL and 16µL, were tested. Inter-/intra-run quantification values when SYTO™ 17 and SYTO™ 64 were added to the assay were compared to those observed when using the standard Quantifiler Trio™ protocol. Intra-run variation of DNA concentration values (using both 11µL and 16µL reaction conditions) for LA and SA targets was approximately 50% lower when SYTO™ 17 and SYTO™ 64 were added. Similarly, intra-run variation improved by 25% and 80% for the Y quantification values when SYTO™ 17 and SYTO™ 64 were added to the assay, respectively. While inter-run comparisons of quantification data when SYTO™ 64 was added (using the 16µL reaction condition) also showed a decrease in LA targets across runs (2.5-fold less), there were major increases in variation across runs for the SA (4.8-fold) and Y targets (6.4-fold). Further, the actual quantification values obtained when either of the SYTO™ dyes were added (using the 11µL reaction condition) were higher than those obtained when the standard Quantifiler Trio™ protocol was used to quantify the same samples. This increase was more than the normal variation that is observed across runs using the traditional protocol, ranging from 10-28% higher with SYTO™ 17 and 18-31% higher with SYTO™ 64, depending on the target. In comparison, normal run-to-run variations observed using the Quantifiler Trio™ kit are less than 10% for each quantification target included in the assay. Fortunately, the increases in quantification values observed when the SYTO™ dyes were added across all targets and not only in targets whose fluorophores overlapped with the spectral emission range of the added dye. Thus, these differences are likely due to the need to further optimize the QuantStudio™ 6 standard curve analysis settings used when each of the SYTO™ dyes are added. Once this issue is mitigated, the integrated HRM/Quantifiler Trio™ assay will be used to analyze the entire preliminary data set of single-source and mixture samples. If prediction accuracies are below 80%, the STR primers used for the HRM targets will be redesigned to better isolate the STR melt range.

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

1. Smith, C. Integration of a high-resolution melt curve STR assay into a commercial quantification kit for preliminary mixture detection. *Int J Legal Med* 2021;(Pending Publication).
2. Dawson, Cruz T. qPCR Genotype Determination and Mixture Detection Using High Resolution Melting Curve Analysis of STR Loci. *NIJ Final Report* 2019.

### **High-Resolution Melt, Real-Time PCR, Mixture Detection**



### **B70 Using Mitochondrial DNA (mtDNA) and the Y Chromosome to Estimate Population Affinity and Region of Origin**

*Andrew Schweighardt, PhD\*, Office of Chief Medical Examiner, New York, NY; Angela Soler, PhD, Office of Chief Medical Examiner, New York, NY*

**Learning Overview:** Attendees can expect to learn about a new concept for predicting the region of origin for an unidentified decedent. For challenging cases with decomposed remains, investigations traditionally rely upon an anthropological assessment, but recently developed and expanded databases for lineage markers have now made it possible to include a genetic component. The DNA-based side of the prediction is easily performed on publicly accessible databases and may be a valuable addition to investigations with an unidentified decedent.

**Impact Statement:** This presentation will impact the forensic science community by outlining a new paradigm for investigations with unidentified decedents. The need for a multidisciplinary approach to this process is underscored by the unique contribution that each makes to the investigation. Individually, the methods used for prediction of population affinity may be potentially inaccurate, but taken collectively, they offer a more holistic approach that draws on the strengths of each.

The EDNAP mtDNA Population Database (EMPOP) and the Y-Chromosome Haplotype Reference Database (YHRD) are the world's largest collections of lineage marker reference profiles for mitochondrial DNA (mtDNA) and Y chromosome haplotypes, respectively. These databases have primarily been used for computing haplotype frequencies but can also offer information about "population affinity" because the reference haplotypes are collected from known locations throughout the world. The population-specific reference samples of EMPOP and YHRD, together with anthropological interpretations and migration history, may assist in the prediction of population affinity for unknown decedents.

In medicolegal investigations, a case with an unidentified decedent deprives family members of answers and may be a barrier to understanding the circumstances of a suspicious death and resolving the case. Predicting population affinity may be helpful for cases in which the decedent is unidentified, because any small clue regarding the decedent may be the key that leads to identification and investigative progress. When decomposition precludes visual assessment, population affinity has traditionally been predicted using an anthropological analysis that evaluates characteristics such as craniometrics and cranial morphology. More recently, a multidisciplinary approach has paved the way for collaborative efforts in which anthropologists and molecular biologists pool their resources. In addition to an anthropological assessment, a DNA-based component can now be included. This has been made possible by databases such as EMPOP and YHRD, which complement each other by providing population-specific samples for maternal (EMPOP) and paternal (YHRD) lineages.

Seventy individuals consented to having their mtDNA profile searched in EMPOP. The findings indicated that the region of origin predicted by EMPOP aligned with the self-identification for 61% of individuals. For almost a third of these individuals, the region of origin predictions was extremely specific and precise, without the inclusion of any other regional predictions.<sup>1</sup> A sample of ten consenting male participants were also recruited to grant permission to search their Y-STR profile in YHRD. The YHRD results were then compared to the participant's own self-identification for the purpose of determining how well the two align. The predicted metapopulation was accurate for all participants, and the specific paternal country of origin was correctly predicted for 30% of the sample.

Both EMPOP and YHRD have been applied to cases of unidentified decedents being investigated by the NYC OCME. Several case studies will be highlighted which illustrate instances where the EMPOP and YHRD results have been utilized with anthropological assessments. The results emphasize the importance of using a multidisciplinary approach to predict population affinity, and the need to understand the benefits and limitations of each method.

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

- <sup>1</sup> Soler, A.; Schweighardt, A.J.; Huynh, L.; Gajewski, C. (2019) Estimating ancestry and region of origin: A multidisciplinary approach to the identification of unknown individuals. *Proceedings of the American Academy of Forensic Sciences, 71<sup>st</sup> Annual Scientific Meeting*, Baltimore, MD. 2019.

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#### **Population Affinity, Haplotype, Unidentified Remains**

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## **B71 Solving Cold Cases With Databases: Considerations for the Application of Advanced DNA Searching Methodologies**

*Ashley Rodriguez\*, RTI International, Research Triangle Park, NC*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the searchable databases associated with familial DNA searching (FS) and forensic genetic genealogy (FGG) and will understand how limitations of these databases can influence the application of these methodologies to forensic investigations.

**Impact Statement:** Familial DNA searching (FS) and Forensic Genetic Genealogy (FGG) are advanced DNA searching methodologies that have demonstrated their value in solving cold case violent crimes. It is necessary for the forensic community to understand that both methodologies are limited by the sample populations within their respective searchable databases in order to avoid the inappropriate application of the technology and possible consumption of evidence.

Both familial DNA searching and forensic genetic genealogy have been used to solve challenging cold cases, yet these techniques differ from each other in the information they can provide as investigative leads based on the limiting factors of the databases they use. This presentation will discuss the differences between these techniques and will recount recent successes to demonstrate the value of these types of searches. The presenters will describe how the database populations and limitations associated with the searchable databases play a role in the type of information that can be obtained, will highlight how the application of these techniques can be streamlined for the identification of missing persons or victims as compared to suspects and will provide suggestions for how stakeholders can evaluate the forensic evidence in a cold case to align it with the technique most likely to provide an investigative lead. Lastly, suggestions will be made for identifying possible challenges when applying these methodologies, solutions to overcome such challenges and how the crime laboratory is a valuable partner when considering these searches.

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**Familial Searching, Forensic Genetic Genealogy, Databases**

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## **B72 FauxDIS: An Interactive Online Forensic DNA Profile Database**

*Ashley Hall, PhD\*, University of Illinois at Chicago, Chicago, IL; Jonathan Bisson, PhD, University of Illinois at Chicago, Chicago, IL*

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**Learning Overview:** After the presentation, attendees will understand the theory and function of a tool that can be used by forensic science educators in reinforcing critical thinking and the scientific method.

**Impact Statement:** The presentation will impact the forensic science community with the demonstration of an online tool useful in educating future forensic scientists.

Forensic Science has captured our collective imagination for generations, whether it be in the medical examiner's room with Quincy, examining blood spatter with Dexter, or in the crime lab with Forensic Files. With the right tools and applications, educators can take advantage of this popularity and use forensic science as a vehicle to teach critical thinking skills and the scientific method, both of which are integral in the collection and analysis of forensic evidence. The forensic scientist makes observations, formulates hypotheses about the probative value of evidence, and tests these educated guesses by submitting crime scene samples to an operational forensic laboratory for analysis. With a DNA profile generated from crime scene evidence, the forensic scientist can conduct direct or indirect database searches in hopes of finding a match and learning the identity of the donor of the questioned sample. The U.S. national DNA database system, CODIS, contains millions of offender DNA profiles, but its use is restricted to authorized operational labs.

FauxDIS is an interactive online forensic DNA profile database that is available to educators for use in learning exercise, such as mock crime scenes, that require students to apply critical thinking skills in the analysis of forensic evidence. The FauxDIS workflow incorporates instrumentation and protocols analogous to those employed in U.S. operational crime laboratories. The database can be used to simulate both direct and indirect profile searches, demonstrating principles of genetics. It also supports experimentation with partial profiles, which can be useful in simulations of degraded and damaged samples commonly found at a crime scene. Further, with a successful database search, random match probabilities can be calculated using either observed or predicted allele frequencies. These experiential exercises teach valuable skills, and the practical experience that students gain may be attractive to potential employers.

FauxDIS currently contains 151 autosomal profiles. Growing the database with additional profiles will increase its utility. DNA profiles will be generated in-house, and will be accepted profiles from other educators, ensuring that the DNA profiles they use in mock crime exercises will be found in the database. FauxDIS is a dynamic entity; it can be expanded to accommodate new marker systems in response to advances in forensic science. In the future, additional indices will include Y-STRs, single nucleotide polymorphisms and massively parallel sequencing data. With this database, the hope is to provide a tool for experiential exercises and contribute to a collaborative network of educators.

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**DNA Database, Forensic Science Education, Critical Thinking**

**B73 Using Mitochondrial DNA (mtDNA) and the Y Chromosome to Estimate Population Affinity and Region of Origin**

*Andrew Schweighardt, PhD\*, Office of Chief Medical Examiner, New York, NY; Angela Soler, PhD, Office of Chief Medical Examiner, New York, NY*

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**WITHDRAWN**

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## **B74 The Interpretation of Y-Chromosome Short Tandem Repeats (Y-STRs) for Missing Persons Cases**

*Jianye Ge, PhD\*, University of North Texas Health Science Center, Fort Worth, TX; Benjamin Crysip, PhD, University of North Texas Health Science Center, Fort Worth, TX; Dixie Peters, MS, University of North Texas Health Science Center, Fort Worth, TX; Meng Huang, PhD, University of North Texas Health Science Center, Fort Worth, TX; Bruce Budowle, PhD, University of North Texas Health Science Center, Fort Worth, TX*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the interpretation methods and guidelines of Y chromosome STRs for complex kinship analysis cases with mutations and/or multiple references.

**Impact Statement:** This presentation will impact the forensic science community by providing more sophisticated interpretation methods and guidelines on Y chromosome STRs for missing person identifications and kinship analysis in general.

Y chromosome STR haplotypes have been used in assisting forensic investigations primarily for identification and male lineage determination. The Scientific Working Group on DNA Analysis Methods (SWGDM) Lineage Marker Committee published interpretation guidelines for Y-STR typing, which provide helpful guidance. However, these guidelines do not address the issue of kinship analysis with Y-STR haplotypes. Because of the high mutation rate of Y-STRs, there are complex missing person cases in which inconsistent Y-STR haplotypes between true paternal lineage relatives will arise and cases with two or more male references in the same lineage and yet differ in their haplotypes. Therefore, more useful guidelines are needed for interpretation of Y-STR haplotype data. Computational methods and interpretation guidelines have been developed specifically addressing this issue, either using a mismatch based counting method or a pedigree likelihood ratio method. However, these methods and guidelines have not been adopted by forensic laboratories, likely because of a lack of specific procedures and software to facilitate analyses.

The Missing Persons Unit under the Center of Human Identification at the University of North Texas Health Science Center (UNTCHI) specializes in the DNA analysis and identification of missing persons cases and processes >50% unidentified human remains of the missing persons cases in the US. It is common within UNTCHI to encounter complex cases that would be better served with enhanced Y-STR interpretation procedures. With the experiences and resources at UNTCHI, more sophisticated interpretation methods and guidelines for Y-STR applications are implemented, which include pairwise comparison with a mismatch-step-based counting method to quickly determine if two profiles are from the same male lineage, and a pedigree likelihood ratio (PLR) based method to evaluate the evidence weight of Y-STR profiles, particularly for complex missing persons cases. A software program is under development and will be validated to facilitate Y-STR haplotype interpretation, and the software will be made accessible to the forensic community free of charge.

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### **Y-STR, Missing Persons, Interpretation**

## **B75 An Evaluation of a 74 Microhaplotypes (MHs) Assay for Kinship Testing in Four Major United States Population Groups**

*Fabio Oldoni, PhD\*, Arcadia University, Glenside, PA; Chiara Della Rocca, MS, Sapienza Università, Rome, Italy; Daniele Podini, PhD, The George Washington University, Washington, DC*

**Learning Overview:** After attending this presentation, attendees will understand the use of microhaplotype (MH) markers in kinship testing.

**Impact Statement:** This presentation will impact the forensic science community by describing the potential use of MHs in addition to conventional STRs as an additional biomarker for kinship analysis.

MHs are new biomarkers consisting of more than two single nucleotide polymorphisms (SNPs) displaying various allelic combinations within an expanse of ~300bp. The multi-allelic nature of MHs makes these alternative loci suitable for massively parallel sequencing (MPS) and more informative than individual SNPs. Forensic applications include human identification, mixture deconvolution and biogeographical ancestry inference. Due to the low mutation rate of SNPs, MHs can also be useful in kinship analysis.<sup>1</sup> This work evaluated the performance of a new MH assay in kinship analysis under different case scenarios.

A newly developed bioassay of 74 MHs was tested on the Ion S5™ sequencing System for kinship analysis. In particular, a large set of families from four distinct U.S. population groups (i.e., 117 Afro-Americans, 68 European-Americans, 105 Southwest Hispanics, and 82 East-Asian Americans) was genotyped.<sup>2</sup> These included both family trios and families with multiple children. Allele frequencies of each U.S. family group were estimated using the recently published U.S. database of 74 MHs.<sup>3</sup> To input MH data and determine the likelihood ratio (LR) distributions under parent-child, full-siblings, half-siblings, and first-degree cousin scenarios, Familias software v.3.2.8 was used.<sup>4</sup> A dataset of 29 short tandem repeats (STRs) from the same U.S. populations obtained from the National Institute of Standards and Technology was also run on Familias and results compared to MH data.

A total of 1,000 simulation tests were performed per each U.S. family group. For parent-child scenarios, the LR distribution curves of parent-child pairs vs unrelated pairs were well separated for both the 29 STRs and 74 MHs, which showed higher median LR distribution values across the four U.S. population groups and stronger support for true related vs unrelated scenarios. Similarly, this was observed in full-siblings scenario vs unrelated case scenario; however, some overlap of the LR distributions were observed with STRs. For half-sibling scenarios, some overlap of the LR distribution curves of half-sibling scenario vs unrelated scenario was observed for both STRs and MHs, although the latter displayed higher median LR distribution values. For first degree cousin scenarios, high level of overlap of the LR distributions were observed for both STR and MH markers.

This study indicated that the 74-MH assay is promising and reliable in clearly solving paternity and full-sibling cases. When testing half-sibling and first-degree cousins versus unrelated case scenarios, some overlaps of the LR distributions were observed, which may lead to inconclusive results. In a nutshell, these results further support the use of this comprehensive and versatile MH tool in solving complex family pedigrees.

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

1. Staadig, A.; Tillmar, A. (2021), Evaluation of microhaplotypes in forensic kinship analysis from a Swedish population perspective, *International Journal of Legal Medicine*, 4,1151-1160.
2. Oldoni, F.; Bader, D.; Fantinato, C., et al. (2020). A sequence-based 74plex microhaplotype assay for analysis of forensic DNA mixtures. *Forensic Science International: Genetics*, 49, 102367.
3. Oldoni, F.; Yoon, L.; Wootton, S., et al. (2020), Population genetic data of 74 microhaplotypes in four major US population groups. *Forensic Science International: Genetics*, 49, 102398.
4. Kling, D.; Tillmar, A.; Egeland, T. (2014). Familias 3-Extensions and new functionality. *Forensic Science International: Genetics*, 13, 121–127.

### **Microhaplotypes, Kinship Testing, Massively Parallel Sequencing**

## B76 Microwave Pre-Processing for Enhanced Rapid DNA Analysis of Forensic Samples

*Nicole Fernandez-Tejero, MSc\*, Florida International University, Miami, FL; Fabiana Taglia, MSc, Florida International University, Miami, FL; Casandra Hernandez-Setser, PhD, Florida International University, Miami, FL; Ling Wang, PhD, Florida International University, Miami, FL; Robert O'Brien, BS, National Forensic Science and Technology Center, Largo, FL; Kevin Lothridge, MS, National Forensic Science and Technology Center, Largo, FL; Bruce R. McCord, PhD, Florida International University, Miami, FL; Steven B. Lee, PhD, Florida International University, Miami, FL*

**Learning Overview:** After attending this presentation, attendees will understand how a microwave can be used to rapidly extract DNA.

**Impact Statement:** This presentation will impact the forensic community by demonstrating how microwave pretreatment extraction can increase allele recovery for conventional DNA analysis and Rapid DNA analysis in low template samples.

In recent years, there have been efforts to develop and implement automated, rapid DNA analysis systems that can process biological samples from swab to profile in 90 minutes. This has proven effective for high-quality samples and known references, but complete DNA profiles are difficult to obtain from less than pristine forensic evidence samples. Since microwaves can disrupt cell membranes, their use prior to rapid DNA analysis provides a quick and inexpensive method to augment the recovery of DNA. We have found that this can be achieved with an off-the-shelf microwave at 300W with a total of 40 seconds run time.

Microwaving samples prior to capillary electrophoresis conventional DNA analysis resulted in a 340% increase in allele recovery from 1% blood, 55% increased recovery from 1% saliva, and 96% increased recovery from 0.5% semen samples. In 1:9 saliva/semen mixtures, the total number of detected alleles increased from 20 to 25 alleles with microwaving, with the minor donor allele peak heights increasing 106% to 130% after microwaving. Rapid DNA processing of mock gun samples produced  $11 \pm 2$  alleles when microwaved in comparison to  $7 \pm 6$  alleles by conventional processing.

We focused on the ability to develop DNA profiles from samples in the stochastic range since this presents one of the main challenges to introducing rapid DNA to routine forensic casework. We empirically determined this was best represented by 0.05% dilutions of saliva where there was > 85% enhancement in allele recovery with microwaving before Rapid DNA analysis. The original sample collection method also profoundly impacts the downstream allele recovery with wet swab collection resulting in 5-6 alleles which increased to 12-13 alleles with dry swab collection. Interestingly, we found that simply removing the original swab from the cartridge (where 7 alleles were developed), allowing it to dry between runs, and then reprocessing permits the recovery of additional alleles when rerun. The microwaved rerun samples resulted in a total of 12 additional alleles vs only 11 in the non-microwaved samples.

A set of samples, sent out to two separate forensic casework laboratories, showed similar enhanced results for microwaved samples. We have developed a simple method that permits calibration of microwave power and sample placement to facilitate the calibration and use of a wide variety of instruments. Overall, our findings demonstrate that results from low copy DNA samples run on Rapid DNA systems can be improved using microwave pre-processing of forensic samples.

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### Microwaves, DNA Analysis, Rapid DNA

## B77 Touch DNA Recovery to Inform Activity-Level Propositions: Fingerprints in the Wild and Their Domesticated Counterparts

Ashley Hall, PhD\*, University of Illinois at Chicago, Chicago, IL; Ray Wickenheiser, DPS, New York State Police Crime Laboratory System, New York, NY; Megan Tyahla, BS, University of Illinois at Chicago, Chicago, IL; Nivida Shete, MS, University of Illinois at Chicago, Chicago, IL

**Learning Overview:** After attending this presentation, attendees will understand the scientific basis informing “activity-level propositions,” addressing how the sample got there, rather than simply who it belonged to. This understanding will improve the attendee’s potential for objective scientific validity as their opinions will be backed by data obtained from well-designed trace DNA transfer experiments. The presented flexible model permits the scientific expert to adapt to a wide variety of potential scenarios to meet defense and prosecution hypotheses, improving their evaluation of evidence, interpretation, opinions, and testimony.

**Impact Statement:** This presentation will impact the forensic community with the presentation of a scientifically neutral basis for evaluating alternate scenarios for explanation of why a suspect’s DNA is located at a crime scene. Opinions based on novel experiments using standardized mock fingerprints and hands will support a model to predict various scenarios posited by either defense or prosecution. Improved data to support the evaluations of hypotheses on transfer will provide improved evaluations, interpretations, and testimony in the field of trace DNA transfer. Improved scientific thought based on data will in turn result in improved justice system outcomes.

Interpreting forensic evidence has long been a challenge for forensic scientists as they are asked to evaluate, interpret and testify upon evidence using experimental data designed to maximize the probative value of evidence. Expert’s opinions include a variety of scenarios representing both prosecution and defense perspective. Alternate scenarios to explain why a suspect’s DNA is found at a crime scene is a common form of defense. Evaluating each scenario provides tremendous value when it can be backed by objective data. Touch samples are becoming increasingly informative sources of this data. In many cases, the strength of the DNA evidence is such that the identity of the donor can’t be reasonably disputed, rather the activities surrounding the DNA transfer become the subject of intense debate. Evaluations of the evidence given the donor’s activities inform “activity-level propositions,” addressing how the sample got there, rather than simply who it belonged to.

DNA transfer and recovery are two factors useful in addressing activity-level questions. Quantification of DNA recovery can be a useful tool in defining primary, secondary, and tertiary DNA transfer events. However, there are numerous variables to consider in a crime scenario and controlling for as many of them as possible is critical to generating informative objective data. First, deposition of a touch sample standard with a known DNA content is necessary to produce quantitative recovery data. A mock eccrine fingerprint containing a known quantity of DNA was previously reported.<sup>1</sup> An improvement on this protocol has been the incorporation of sebaceous components, such as amino acids and lipids, into the mock (domesticated) fingerprint chemistry. Using the domesticated sebaceous fingerprint, DNA recovery can be used to more closely approximate the behavior of fingerprints in the wild.

Next, to normalize DNA delivery in the primary transfer, a mock hand has been optimized. Three artificial epidermal formulations were evaluated: (1) a water-responsive, gelatin-based model; (2) a Lorica leather surrogate; and (3) a mixture of polyvinyl alcohol and polydimethyl siloxane.<sup>2-4</sup> The artificial skin was constructed on a nitrile glove supported on a plaster cast of the researcher’s hand. To complete a transfer, the researcher put on the epidermis-glove, a domesticated fingerprint was placed on the skin, and it was transferred to a different surface. DNA recovery was quantified by real-time PCR. By controlling for DNA deposition variables, DNA recovery can be used to inform activity-level propositions.

### Reference(s):

1. Tang, J.; Ostrander, J.; Wickenheiser, R.; Hall, A. Touch DNA in forensic science: The use of laboratory-created eccrine fingerprints to quantify DNA loss. *Forensic Sci Int: Synergy* 2020;2:1-16
2. Dąbrowska, A.; Rotaru, G.; Spano, F.; Affolter, Ch.; Fortunato, G.; Lehmann, S.; Derler, S.; Spencer, N.D.; Rossi, R.M. A water-responsive, gelatine-based human skin model. *Tribology International* 2017;113:316-322.
3. Cottenden, D.J.; Cottenden, A.M. A study of friction mechanisms between a surrogate skin (Lorica soft) and nonwoven fabrics. *J Mech Behav Biomed Mater* 2013;28:410-26.
4. Morales-Hurtado, M.; Zeng, X.; Gonzalez-Rodriguez, P.; Gonzalez-Rodriguez, P.; Ten Elshof, J.E.; van der Heide, E. A new water absorbable mechanical Epidermal skin equivalent: The combination of hydrophobic PDMS and hydrophilic PVA hydrogel. *J Mech Behav Biomed Mater* 2015;46:305-17.

### Touch DNA, Mock Fingerprint, Activity-Level Propositions



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## **B78 A Responsive Academy Seeing the Modern Forensic World Through the Lens of Its History: Lessons Learned From the Pioneering Jan Bashinski**

*Sandra Sachs, PhD\*, Oakland Police Department, Oakland, CA*

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**Learning Overview:** The importance of the modern forensic world keeping history in mind when moving forward.

**Impact Statement:** Historical issues laboratories faced remain topics that forensic organizations wrestle with to this day.

The Modern Forensic World is faced with enormous challenges: Sexual Assault Kit backlogs, maintaining accreditation, inadequate funding, onboarding emerging technology, legal challenges, training analysts, publishing/sharing research, certification, scope of expertise, standards development and more.

It is worth taking a look back through the last five decades to see where the field of forensic science has been. Indeed, "A generation which ignores history has no past and no future."<sup>1</sup>

In the 1970s a heightened awareness about Sexual violence against women was emerging. Jan Bashinski, the first woman hired at the Oakland Police Department Criminalistics Laboratory, and her collaborators moved the needle by investigating ways in which this type of evidence could be handled and processed to its fullest potential. She conducted this work in the tiny municipal laboratory servicing the residents of Oakland. Jan initiated research relationships with local talent at UC Berkeley to make this happen. She did this with no extra funding for such efforts.

Jan received master's degree in Criminology from UC Berkeley in 1974 for work evaluating Gunshot Residue for lead.

In 1977 Jan inherited the helm of the Oakland PD as the first female forensic laboratory director in the State of California. In 1983, she led the charge to have the Oakland Laboratory become the first in California and the fourth in the nation to be accredited.

In addition to these accomplishments, Jan provided leadership in a multitude of ways:

- Early adopter and advocate of Accreditation through ASCLD/LAB
- Strong regional forensic science association (CAC) attendance and collaboration
- President in 1977
- Presenter of technical papers on many occasions
- National leadership
- AAFS Criminalistics Section Chair, 1984-85
- Certification of forensic scientists – CAC which assisted to launch ABC
- Generalist approach
- Founded the California State DNA Laboratory Program and DNA Database
- DNA Laboratory in Richmond named after her through CA legislation
- Cold hit program – sought and received funding for California municipal labs
- More than 10,000 cases reopened and 366 cold hits were achieved
- Mentorship
- Training forensic practitioners
- Oversaw the California Criminalistics Institute (CCI)

This presentation is not intended to be hagiographic. Instead, it is simply to set the stage of the special session for discussion and reflection on the ways past figures in our forensic profession blazed trails and, in so doing, what lessons can be learned about how to proceed tackling the innumerable emerging issues at our collective forensic door: Recognition of vicarious trauma, mitigation of bias, forensic management certification, black box studies to address error rates, next generation sequencing, cutting edge instrumentation, method development. Which will we tackle? How will we do so?

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

- <sup>1</sup>. Robert Heinlein. *Time Enough for Love*. 1973.

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### **Laboratory Issues, Certification/Accreditation, Working Group Standards**

## B79 The Important Role of Generalists in the Modern Forensic Science World of Specialists

JoAnn Buscaglia, PhD\*, FBI Laboratory, Quantico, VA; Jack Hietpas, PhD\*, Microtrace, Elgin, IL; Peter De Forest, DCrim, John Jay College of Criminal Justice, New York, NY

**Learning Overview:** Attendees will learn the value of generalists in meeting and surpassing the challenges in the modern forensic science world, which is overwhelmingly dominated by specialists.

**Impact Statement:** In keeping with the conference theme, this presentation will impact the forensic science community and honor the legacy of Jan Bashinski by exploring the value of generalists and their impact on the modern forensic science world, particularly in the areas of research, mentoring, quality assurance, and administration.

Historically, the role of the criminalist has been as a forensic science generalist – a competent scientist with sufficient breadth and depth to define the appropriate investigative scientific questions, and to recognize, recover, examine, and interpret traces. However, as early as 1969, Kirk reported the trend toward specialized scientists confined to the laboratory “without the basic principles of scientific crime investigation” – a trend that has continued up to today with few exceptions.<sup>1</sup> De Forest has tirelessly advocated for “recapturing the essence of criminalistics” and the importance of the role of the scientist at the crime scene.<sup>2</sup> Similarly, an international group of scientists recently presented the Sydney Declaration, which defines forensic science as “... a case-based (or multi case-based) research-oriented endeavour (sic) using the principles of science to study and understand traces....”<sup>3</sup> Although there is certainly a need for specialists for highly technical methods and advanced instrumentation, there remains a need for the proactive generalist not only in scientific crime investigation but also in research, quality assurance, mentorship, and administration of crime laboratories.

In keeping with the spirit of the historical criminalist, forensic scientists should strive for a forward-thinking, research orientation regardless of whether their work is scene- or laboratory-based. One can be highly specialized in one or more areas yet still be a generalist by keeping casework and research interests wide and asking the appropriate questions that are based on sound science. As technological advancements continue, there is no doubt that certain areas will become more highly specialized, requiring more specialists to analyze a single item of evidence. However, in such a situation, the role of the generalist will be even more critical – in ensuring that the right questions are asked, the appropriate tests are conducted to answer them, and the significance of their results are properly interpreted within the context of the case. Without this holistic understanding, there may very well be highly specialized tests done that give the *right* answers to the *wrong* questions, wasting precious (evidentiary, financial, and human) resources and failing to serve the criminal justice system and society as a whole.

Although research in support of the forensic sciences often requires highly specialized knowledge in a particular area, it is also likely multidisciplinary, for which it is most appropriate for a generalist to lead the research effort. “Chance favors the prepared mind” – a quote attributed to Pasteur – exemplifies the important role of the generalist in research; success in research requires grounding in the scientific approach to problem-solving. Similarly, successful implementation of research into practice often requires a broad knowledge of the forensic sciences and criminal justice system as a whole – reasons to have a strong and broad educational foundation in science and applications in Criminalistics.

This session honors Jan Bashinski, who was wholeheartedly a generalist – forward-thinking, problem-solving, and research-oriented. From GSR and drug chemistry to forensic biology, to accreditation and laboratory management, Jan’s education and knowledge in Criminalistics and her generalist approach provided the strong foundation for her diverse career, which had a profound impact on forensic science and the criminal justice system.

### Reference(s):

1. Kirk, P.L. Criminalistics at the crossroads. *The Criminologist*. 1969;4(11):35–42.
2. De Forest, P.R. Recapturing the essence of criminalistics. *Sci Justice*. 1999;39(3):196–208. [https://doi.org/10.1016/s1355-0306\(99\)72047-2](https://doi.org/10.1016/s1355-0306(99)72047-2).
3. *Sydney Declaration*. <https://iafs2023.com.au/cms/wp-content/uploads/IAFS-2023-Sydney-Declaration-18-May-2021.pdf> (accessed 26-August-2021).

### Generalist, Criminalist, Specialist

### **B80 Taking the Lead: Jan Bashinski and the Development of Modern Analytical Methods for the Chemical Detection of Gunshot Residues**

*Michelle D. Miranda, PhD\*, Farmingdale State College, SUNY, Farmingdale, NY*

**Learning Overview:** After attending this presentation, attendees will have learned about the history, chemistry and present approaches to GSR analysis, specifically the detection of lead residues using the sodium rhodizonate test. During the course of the presentation, attendees will learn about Bashinski's contributions to GSR analysis, which includes techniques still routinely employed in crime laboratories conducting GSR analysis.

**Impact Statement:** The presentation will impact the forensic science community by providing a review of the history and chemistry of GSR analysis, the contributions of Bashinski to the discipline, and their continued relevance today.

GSR examinations consist of visual, microscopical, chemical and spectroscopic examinations of the residues that result from the discharge of a firearm and ammunition. The major contributors to the deposition of GSR on a surface proximal to a discharge event include the projectile (bullet, shot), propellant (gunpowder), and the primer. Primer GSR (pGSR) analysis is largely concerned with examinations of "shooter's hands" samples utilizing SEM-EDS to detect inorganic residues derived from the primer (barium, antimony, and lead). pGSR interpretations address whether or not an individual may have recently fired, handled, or was in the vicinity of a discharged firearm. Supplemental analysis for the detection of organic components (propellant) may be conducted in conjunction with pGSR testing. Alternatively, the broad analysis of firearm discharge residues is often associated with color tests for the residues exiting from the muzzle end of the firearm and being subsequently deposited, often in a pattern, on a target or intervening object. The sequence of color tests is selected in order to detect the presence of both organic (nitrites) and inorganic (copper, lead) traces. Detection and interpretation of these residues can aid in determining the passage of a bullet; the discharge of a firearm; the approximate distance between the shooter and the target (muzzle-to-target distance determinations); and the maximum distance at which a particular firearm with particular ammunition will cease to deposit residues on a target (maximum distance determinations).

Presently, the most common color tests employed in such GSR testing are the dithiooxamide test for copper, the modified Griess test for the detection of nitrites, and the sodium rhodizonate test for lead. This presentation will focus on the history and chemistry of the sodium rhodizonate test and the research of Jan Bashinski in the 1970's which resulted in the technique referred to as the *Bashinski Transfer Method*. The sodium rhodizonate test has its roots as a general spot test for trace metal detection in analytical chemistry, and was eventually recognized for its application to matters of forensic significance—notably, the colorimetric detection of lead residue resulting from the passage of a bullet. The utility of the sodium rhodizonate test extended beyond a spot test indicating presence or absence of lead to a test that could provide information *in situ* about lead deposition based on the pattern deposited on the target, lending itself to making approximations about the distance between the firearm and the target surface. Bashinski *et al.* reviewed the chemistry and methodology of sodium rhodizonate in a forensic context to provide the forensic community with a test that was useful and robust for distance determinations, supporting the utilization of the sodium rhodizonate test for routine GSR examinations in crime laboratories.<sup>1</sup> Moreover, Bashinski *et al.* brought attention to the importance of well-informed approaches to interpreting GSR results.<sup>1</sup> Despite modern advances in technology and application of spectroscopic and chromatographic methods to identify and classify gunshot residues, the analytical methods and interpretation considerations addressed by Jan Bashinski in her research endeavors are still of critical importance to how forensic scientists approach GSR cases presently.

**Reference(s):**

- <sup>1</sup> Bashinski, J.; Davis, J.; Young, J. (August 1974). Detection of Lead in Gunshot Residues on Targets Using the Sodium Rhodizonate Test. *AFTE Journal* 6(4), 5-6.

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#### **Gunshot Residue (GSR), Sodium Rhodizonate, Shooting Reconstruction**

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## **B81 Jan Bashinski's Progressive Approach to Crime Lab Management: The Development of the Systematic Approach for the Analysis of Semen Evidence From 1978 to 1985**

*George Sensabaugh, DCrim\*, University of California Berkeley, Berkeley, CA; Edward Blake, DCrim, Retired, Oakland, CA*

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**Learning Overview:** This presentation honors Jan Bashinski's contribution to the development of a systematic approach to the analysis of evidence in sexual assault cases.

**Impact Statement:** This presentation illustrates how crime labs can benefit from creating an environment marshaling the efforts of the public, private, and academic sectors to work together on forensic problems of the day.

Sexual violence against women emerged in the 1970's as a major public issue: police investigations of rape were often superficial, prosecution rates were low, and conviction rates even lower. Two consequences with immediate forensic relevance were calls for better treatment of victims in emergency rooms and greater utilization of physical evidence in supporting investigations and prosecutions.

Jan was director of the Oakland Police Department Criminalistics Laboratory as these issues were coming to a head. Jan recognized that the path to increasing the crime lab's contribution to the utilization of physical evidence began with better procedures for the collection of evidence in the ER and preservation of evidence prior to analysis in the lab. She also appreciated a need to improve the information yield provided by the then-current protocols used for the analysis of sexual assault evidence.

To address these objectives, Jan initiated a working consortium involving her lab with an academic research lab and a private practitioner, both with research experience with sexual assault evidence. She was successful in obtaining research grants to support the work of the consortium and administered the grants with a firm hand to keep the research objectives in sight.

As the potential value of sexual assault evidence came to be appreciated in the 1970s, the collection of evidence from sexual assault victims typically involved collection of vaginal swab samples according to clinical sterile practice: wet swabs were placed in sealed tubes for submission to the crime laboratory. These conditions were hypothesized to lead to degradation of the genetic markers of forensic interest, specifically the ABO blood group substances and polymorphic enzyme markers that might be present. Basic research on protein and blood group stability demonstrated that both markers were very stable in the dried state. Follow-up research demonstrated that markers on dried vaginal swabs persisted longer than on wet vaginal swabs even when frozen. Finally, it was demonstrated that rapid drying was better than drying under ambient conditions. This led to the development of the drying box, a box to be placed in the ER to dry freshly collected swabs in a stream of moving air. This body of research laid the foundation for the current practice of storing biological evidence in the dry state to maintain marker stability.

By the end of the 1970's, the analysis of vaginal swab evidence in sexual assault cases was limited to presumptive testing for the presence of semen and/or detection of sperm by microscopy followed by, if semen was detected, genetic testing for ABO blood group antigens and the genetically polymorphic enzymes phosphoglucomutase (PGM) and peptidase-A (PepA). Because these genetic markers are also expressed in vaginal fluids, genetic testing of vaginal swab extracts is nominally informative as to the semen donor only when markers foreign to the victim's types are detected. To address this limitation, a systematic scheme for the analysis of semen evidence was devised which entailed estimating the amount of semen present in a vaginal swab extract by quantitative acid phosphatase analysis supported by microscopic assessment of the sperm content. This quantitative variable could then guide the interpretation of genetic typing results: genetic types detected under this scheme can be attributed to the semen donor if the semen content of the vaginal swab extract exceeds the upper limit of the genetic markers normally present in vaginal fluids.

These examples illustrate Jan's progressive approach to crime laboratory management - by creating an environment in which the efforts of public, private, and academic sectors were marshaled to work together, significant progress was made in addressing an important forensic problem of the day.

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**Criminalistics, Sexual Assault, Crime Lab Management**

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### **B82 Examining the Analytical Threshold (AT) Methods Used in Interpreting Capillary Electrophoresis (CE) - Based Short Tandem Repeat (STR) Profiles**

*Sarah Riman, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Hari Iyer, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Peter Vallone, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending this presentation, attendees will gain valuable insight into the methods used to select analytical thresholds (ATs) and the impact of AT values on downstream analysis.

**Impact Statement:** This presentation will benefit the forensic science community by discussing a framework to assess an AT(s) and their impact on casework interpretations.

Continuous probabilistic genotyping software (PGS) model true allelic peaks with their associated PCR byproducts. PGS also model drop-in and drop-out events. Most PGS require STR profile information with an AT pre-applied to remove baseline noise and similarly setting these AT values within the PGS to model drop-in and drop-out peaks during Likelihood Ratio (LR) assignment. Therefore, setting an AT to help distinguish an allelic signal from electronic noise remains a critical step and is still to be determined until noise models are incorporated into PGS.

There is no 'perfect' AT for all situations. This is the reason there are different methods and equations that provide guidance to establish AT and none of the formulas are normative. Higher AT values will prevent erroneously labeling non-alleles as alleles decreasing drop-in events (false positives). However, a high AT value can lead to drop-out events (false negatives). Therefore, an established AT value should provide a satisfactory balance point between drop-ins and drop-outs.

Here, we first give an overview of: (1) different factors that affect the peak heights of both the noise and true alleles, and (2) various published methods and equations that are used to determine AT values. We then examine the performance of these different AT approaches and their effect on: (1) rates of false positives (false labeling of electronic noise peaks as true alleles), (2) true positives (correctly labeling peaks as true alleles), (3) number of contributor (NOC) assessment, and (4) LR assignment. To illustrate these ideas, we derive AT using single-source profiles of varying DNA quality and DNA quantity obtained from the GlobalFiler 29 cycles 15s PROVEDIt dataset. We then evaluate the performance of ATs derived from single-source samples when applied to unknown samples by using a set of PROVEDIt mixture profiles of varying quality, quantity, NOCs, and mixture ratios.

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#### **Analytical Thresholds, DNA Mixture, True and False Positive Rates**

## **B83 Lessons Learned From a 40-Year Career in Forensic Science**

*Barry Fisher, MS, MBA, LAPD Crime Lab, Retired, Indio, CA*

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**Learning Overview:** There is no substitute for experience. This presentation relates the professional experiences of a senior forensic scientist that may aid younger professionals.

**Impact Statement:** The biblical statement that nothing is new under the sun is partially true in a forensic science career. No two days or cases are ever exactly alike. Drawing on the experience of senior practitioners can give forensic scientists newer to the profession ideas of how to handle situations unfamiliar to them or perhaps that they have never considered.

During my career, I learned a number of useful qualities that can guide forensic scientists. These include:

- Forensic science is not static and requires continuing education.
- Be skeptical. Don't believe the first explanation you develop when obtaining from tests.
- Always consider alternative theories even when presented by adversaries.
- Add as many professional colleagues as you can from all areas and cultivate those individuals.
- Work to improve your communication skills and consider your audience when explaining technical matters.
- Join and become involved in professional societies. Attend conferences.
- When able, present papers at professional conferences and publish your work in peer-reviewed journals.
- When a superior asks for a volunteer to take on a project, volunteer.
- Remember, that when life hands you lemons, make lemonade.
- Maintain a positive attitude.
- Be willing to say, "I don't know." It is impossible to be an expert in all things. Ask for assistance.
- Be willing to assist subordinates when possible.
- Be honest and ethical in all your professional conduct.
- Learn the importance of outreach to groups such as the press, crime victims, senior personnel, judges, and lawyers.
- Keep a journal.

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### **Mentoring, Experience, Coaching**

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## **B84 Fixing Forensic Science by Learning How to Learn from Our Mistakes**

*Brian Gestring, MS\*, 4n6Services, Guilderland, NY*

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**Learning Overview:** Attendees will learn about failure analysis in forensic science and how to improve their response to when things go wrong.

**Impact Statement:** This presentation will highlight a critical weakness in how forensic science is practiced and provide a phased implementation plan describing how to fix it.

The introduction of Forensic Science has forever changed the criminal justice system. Despite how good forensic scientists are at their respective disciplines, inexplicably, they are bad at looking at themselves and learning from when things go wrong with the work they do.

In part, this is due to the many divergent educational paths that lead to a career in forensic science. Examiners can enter the field with a forensic science degree, a traditional science degree, or in some cases, no degree at all. While there is a voluntary accreditation available for academic programs in forensic science, there is no requirement that any failure analysis methodology be discussed.

This weakness is further compounded by existing practitioner's lack of awareness and training. For the most part, forensic science did not start to embrace root cause analysis until the greater forensic community started to migrate to the international accreditation standards (ISO/IEC 17025:2005). 17025 required a process of root cause analysis and corrective action, but there was no training requirement for the forensic scientists who had to develop or carry out these procedures for their laboratory.

To this day, there is no requirement for forensic scientists working in accredited laboratories to receive any root cause analysis training. As a result, the experience levels of the people charged with this responsibility varies from some external training to no formal training at all.

The challenges faced by accredited laboratories are dwarfed in comparison to their non-accredited counterparts who essentially have no requirements at all. As of this writing there is no universal requirement for government run forensic laboratories to be accredited despite calls for this for over a decade.

Lack of formal guidance and training on failure analysis combined with immersion in the adversarial system of justice often leads to rushed assignments of blame and the development of "quick fixes." Failing to understand the full scope and true nature of a problem before coming up with a solution virtually guarantees that the problem will occur again. The apprehension that comes from having no "safe space" to discuss failures often leads to silos of information precluding even other sections of the same laboratory from learning from a mistake, let alone other laboratories or the broader forensic community.

As daunting as this sounds, it is not too late to recognize this Achilles heel and take steps to address this critical weakness. This presentation will outline the current challenges associated with this vulnerability and describe a phased implementation plan demonstrating a path to not only learn from the mistakes that we make, but those of others within the field.

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### **Root, Cause, Analysis**

## B85 Reclaiming Forensic Science: A Closer Look at the Sydney Declaration

*Michelle D. Miranda, PhD, Farmingdale State College, SUNY, Farmingdale, NY; Sheila Willis, PhD, Leverhulme Research Center for Forensic Science, Dundee, United Kingdom; Patrick Buzzini, PhD\*, Sam Houston State University, Huntsville, TX; Rebecca Bucht, PhD, NBI Forensic Science Laboratory, Vantaa, Finland*

**Learning Overview:** After attending this presentation, attendees will learn about the collaborative effort to define forensic science and articulate its fundamental principles in an effort to unify and improve the status and application of forensic science.

**Impact Statement:** The presentation will promote and encourage discussions concerning the fundamental principles of forensic science in order to effectively address education and training, research and development, laboratory practices, and quality of forensic science services.

*Forensic science, forensic sciences, forensics.*

*Forensic scientists, criminalists, generalists, specialists, academics, practitioners, “experts,” “others.”*

*Prosecutors, defense attorneys, judges, law enforcement officers, administrators, policymakers.*

*Who are we? What do we do? Who (and what) defines what we are and what we do?*

*Where have we been? Where are we going? What is our history and what is our future?*

In 2021, an international group of forensic scientists drafted and presented “the Sydney Declaration” in response to years of discussions and debates concerned with fundamental matters related to forensic science.<sup>1</sup> Many of these conversations arose from publications and media accounts addressing the effectiveness and reliability of forensic science. The group opted to approach forensic science from its foundation, thereby establishing fundamental principles with the goal of unifying the purpose of forensic science in order to effectively address matters of education and training, research and development, and laboratory operations as forensic science moves into the future.

This presentation begins with a brief history of criminalistics and is then divided into two sections. First, aligned with the principle that forensic science relies on scientific knowledge and logical reasoning, a discussion of scientific methodology is presented and expanded upon by considering the importance of observation and inferential reasoning within forensic science education and training. The second section emphasizes the principle that forensic science requires meaning in context, which necessitates knowledge of the nature of traces.

A core element of the Sydney Declaration is the trace, or vestige of an activity that occurred in the past under uncontrolled conditions. Traces undergo a process that qualifies them to a status of evidence. This process requires a rigorous methodology that goes beyond the deployment of specialized techniques to include defined scientific principles and human cognition. A reliable methodology intended to determine the contributing value of traces recovered in a case should include the proper use of the scientific method, but where does the scientific method fit in practice? At the laboratory? At the crime scene? Who defines the working hypotheses? Is the scientific method integrated into the transition of traces from the scene to the court, and through the lab? Moreover, can the scientist communicate their observations, interpretations and reasoning processes to others?

Forensic science has been the subject of much criticism in recent years, making it easy to imagine that its use causes more problems than it solves. Yet even the most ardent critics could not imagine a criminal justice system without physical evidence. We need to acknowledge that context has an impact on the scientific process and that findings do not have a stand-alone value. Relevance is sometimes considered to be the concern of the court or the lawyers and not the scientist. But, if the science is to make a useful contribution to an investigation, the knowledge the scientist has on trace activity, persistence and detection needs to be made available to guide scientific methodology and assigning meaning to the results obtained. Forensic science takes some norms from other disciplines, but it is a mistake to confine the contribution to the analytical results of comparisons without consideration of the nature of the source, trace and environment in the context of the case.

### Reference(s):

<sup>1</sup>. *Sydney Declaration*. 2021. <https://iafs2023.com.au/cms/wp-content/uploads/IAFS-2023-Sydney-Declaration-18-May-2021.pdf>.

### Trace, Context, Sydney Declaration



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## B86 Case Contextual Information and Its Variation Among Forensic Laboratories

*Taylor Hopkins\**, Duquesne University, Pittsburgh, PA; *Lyndsie Ferrara, PhD*, Duquesne University, Pittsburgh, PA

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**Learning Overview:** The goal of this presentation is to inform attendees about the information that different forensic disciplines and laboratories collect through evidence submission forms as well as their established bias mitigation techniques.

**Impact Statement:** This presentation will impact the forensic science community by cultivating a continuous dialogue about the impact of contextual bias within the workplace.

One of the challenges in the modern forensic science world is acknowledging and mitigating bias in all of its forms. Contextual bias, in particular, is a challenge as it can unintentionally cause scientists to let case contextual information guide their decisions as opposed to the actual evidence. This bias can arise simply through the use of laboratory submission forms and a study concluded that such forms could contain irrelevant information relating to the examination required.<sup>1</sup> This project investigated the amount of information requested by forensic laboratories, via their submission forms, and how they vary between forensic disciplines.

A survey was created asking participants about their laboratory, laboratory submission form, and laboratory procedures as it relates to case contextual information and how it is transmitted. Participation was completely voluntary and not all questions had to be completed to submit it. The survey was transmitted via email to accredited forensic-testing laboratories according to the ANSI National Accreditation Board (ANAB) website and sent out to members of several regional and national forensic organizations including the American Academy of Forensic Sciences Criminalistics section. Data was collected during the summer of 2021.

A variety of disciplines and laboratories were represented in this data set including forensic biology, chemistry, and firearms/toolmarks. The majority of participants indicated their job position as an analyst as opposed to a laboratory administrator. Most participants also answered that their laboratory used a general laboratory submission form instead of a discipline-specific form. Of the items listed on the survey, the top four reported items on the laboratory submission form were investigating officer, evidence description, suspect name, and offense type. Responses showed that while many laboratories implement bias training, there are still many laboratories that do not have a protocol in place to guard an analyst from potential biasing effects of case contextual information. Overall, the data provided a comprehensive outlook on information collected through evidence submission forms and what different laboratories and disciplines are doing, or not doing, to mitigate the effects of contextual bias.

### Reference(s):

- <sup>1</sup> Gardner, B.O.; Kelley, S.; Murrie, D.C.; Blaisdell, K.N. Do evidence submission forms expose latent print examiners to task-irrelevant information? *Forensic Science International* 2019, 297, 236-242.

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### Bias, Contextual Information, Submission Form

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### **B87 The Development of an *In Situ* Sampling Interface for Thermal Desorption Direct Analysis in Real-Time Mass Spectrometry (TD/DART<sup>®</sup>-MS) Analysis of Ignitable Liquid Residues (ILRs) in Fire Debris Samples**

*Briana Capistran, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD; *Matthew Staymates, MS*, National Institute of Standards and Technology, Gaithersburg, MD; *Edward Sisco, PhD*, National Institute of Standards and Technology, Gaithersburg, MD

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**Learning Overview:** After attending this presentation, attendees will be familiar with a new sampling chamber designed for ILR analysis *via* (TD)-DART<sup>®</sup>-MS to achieve direct sampling, ionization, and detection of sample components.

**Impact Statement:** This presentation will impact the forensic science community by providing a sampling tool for ILR analysis that eliminates sample preparation procedures and enables *in situ* sampling of volatile components.

After attending this presentation, attendees will be familiar with a new sampling chamber designed for ILR analysis *via* (TD)-DART<sup>®</sup>-MS to achieve direct sampling, ionization, and detection of sample components. This presentation will impact the forensic science community by providing a sampling tool for ILR analysis that eliminates sample preparation procedures and enables *in situ* sampling of volatile components. The identification of ignitable liquid residues (ILRs) in fire debris samples can serve as an indication of an intentionally set fire. Gas chromatography-mass spectrometry (GC-MS) is the standard method for ILR analysis because of its ability to separate and identify individual sample compounds. Such analysis requires sample preparation procedures (e.g., passive-headspace extraction), which can be time-consuming (up to 24 hrs), involve the risk of competitive adsorption, and require the use of hazardous solvents (e.g., carbon disulfide). Additionally, GC-MS analysis times can be lengthy (e.g., 30 – 60 min per sample), and ILR identifications can be challenging due to the presence of substrate interferences in the resulting chromatograms and extracted ion profiles. Direct Analysis in Real-Time Mass Spectrometry (DART<sup>®</sup>-MS) has become increasingly popular in forensic laboratories because of the ability to rapidly analyze samples in their native states.

The identification of volatile and nonvolatile compounds is also possible using DART<sup>®</sup>-MS, which is advantageous for identifying nonvolatile fuel additives that are otherwise undetectable using GC-MS. Coupling DART<sup>®</sup>-MS with temperature-programmed Thermal Desorption (TD) compensates for the lack of separation component and provides time-resolved mass spectra for particularly volatile samples, such as ILRs in fire debris. Use of TD has also shown to improve sensitivity and ionization efficiency. Thus, the use of (TD)-DART<sup>®</sup>-MS for ILR analysis eliminates time-intensive sample preparation, reduces the risk of competitive adsorption and exposure to hazardous solvents, and minimizes sample and data analysis times. However, there exists a need for a sampling chamber to accommodate for *in situ* sampling from commonly used sample containers (e.g., paint cans). This work presents a newly developed TD interface amenable to ILR analysis that enables direct sampling of compounds and introduction into the mass spectrometer. The sampling chamber has been constructed to accommodate sample containers of varying sizes. Containers are loaded into the chamber and enclosed with temperature-controlled heating elements for homogeneous heating of the entire sample unit. A temperature program is used to desorb compounds with a wide range of volatilities. Subsequent ionization of desorbed compounds and direction into the mass spectrometer is achieved using a heated junction. Optimization of programmed heating parameters, such as initial and final temperatures, hold times, and ramp rates, was performed specifically for ILR analysis to enhance spectral reproducibility, especially for highly volatile compounds. A range of samples was used to assess the efficacy of the sampling chamber, including standard test mixtures of n-alkanes and aromatic compounds (common ILR components) and simulated fire debris samples.

This work will introduce a novel sampling chamber for (TD)-DART<sup>®</sup>-MS analysis of fire debris samples in their original sample containers. The development of such an interface not only eliminates sample preparation and increases sample throughput but enhances sensitivity and reproducibility for ILR identification in highly complex samples.

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#### **Fire Debris, DART<sup>®</sup>-MS, Thermal Desorption**

## B88 A Sensitivity Analysis of Dynamic Vapor Microextraction with Simulated Fire Debris

Jennifer Berry\*, National Institute of Standards and Technology, Boulder, CO; Mary Gregg, National Institute of Standards and Technology, Boulder, CO; Amanda Koepke, National Institute of Standards and Technology, Boulder, CO; Chris Suiter, National Institute of Standards and Technology, Boulder, CO; Reta Newman, Pinellas County Forensic Laboratory, Largo, FL; Kavita Jeerage, National Institute of Standards and Technology, Boulder, CO

**Learning Overview:** Attendees will learn about the application of dynamic vapor microextraction (DVME) to the extraction and concentration of ignitable liquid (IL) from simulated fire debris, its challenges, and how sensitivity analysis can be used to evaluate this emerging forensic method.

**Impact Statement:** This presentation will impact the forensic scientific community by describing a sensitivity analysis of dynamic vapor microextraction (DVME), an alternative headspace concentration method that may find application in fire debris analysis. DVME employs a safer solvent than typical headspace concentration methods and vapor distortion can be monitored; however, the relative influence and interaction of sample and instrumental variables remains unexplored. Challenges associated with sample packaging will also be addressed.

Debris from structural fires is typically investigated for ignitable liquid (IL) residue using headspace concentration methods. The most common method in the United States is passive concentration onto activated carbon strips (ACSs), which requires the use of carbon disulfide, a dangerous neurotoxic solvent, and is known to have challenges with vapor distortion.<sup>1,2</sup> Dynamic vapor microextraction (DVME), in which a carrier gas pushes vapors from the sampling container headspace through a chilled adsorbent capillary, is a potential alternative. The adsorbent phase of the capillary, along with the cold temperature, enhances the trapping efficiency of vapors and stabilizes reactive species in the sample. DVME was originally developed to identify compounds in the headspace of low volatility explosives and has been applied to the extraction of ILs including gasoline and diesel fuel in preliminary studies.<sup>3-5</sup> This emerging method has promising features for fire debris analysis including the use of a relatively benign elution solvent (acetone) and the ability to monitor vapor distortion. However, the current DVME procedure involves many high-precision controllers (e.g., to control the inlet flowrate and the capillary temperature) using equipment not standard in forensic laboratories. Furthermore, the relative effect of many sample and instrumental factors on the resulting IL extraction remains unknown.

To address this research gap and assess potential instrumental requirements, a sensitivity analysis was conducted with 5 sample and 6 instrumental factors. Simulated fire debris was created by adding weathered gasoline (50% or 80%) to a 32 oz glass sampling container partially filled with burned or unburned wood chips; some samples were spiked with water. DVME was used to collect vapors from the headspace of these containers, varying the adsorbent phase, oven temperature, capillary trap temperature, inlet flowrate, and collection volume. Collected samples were eluted with acetone, analyzed using gas chromatography with mass spectrometry, and compared to reference samples using covariance mapping of the retention time-ion abundance data matrix.<sup>6</sup> Statistical analysis was conducted to determine significant first-order effects and interactions between variables.

This study is an essential step in validating the DVME method for application in forensic practice. Instrumental factors that significantly influence extraction of IL vapors direct future research on optimization, while non-significant instrumental variables identify elements of the procedure that may be modified to facilitate laboratory adoption. The results also provide a better understanding of the robustness of the DVME method to realistic differences in sample composition.

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2. Lentini, J.J.; Armstrong, A.R. Comparison of the Eluting Efficiency of Carbon Disulfide with Diethyl Ether: The Case for Laboratory Safety. *Journal of Forensic Sciences* 1996, 42 (2), 307-311.
3. Lovestead, T.M.; Bruno, T.J. Trace Headspace Sampling for Quantitative Analysis of Explosives with Cryoadsorption on Short Alumina Porous Layer Open Tubular Columns. *Analytical Chemistry* 2010, 82, 5621-5627.
4. Nichols, J.E.; Harries, M.E.; Lovestead, T.M.; Bruno, T.J. Analysis of Arson Fire Debris by Low Temperature Dynamic Headspace Adsorption Porous Layer Open Tubular Columns. *Journal of Chromatography A* 2014, 1334, 126-38.
5. Harries, M.E.; Wasserman, S.S.; Berry, J.L.; Jeerage, K.M. Characterization of a Headspace Sampling Method with a Five-Component Diesel Fuel Surrogate. *Forensic Chemistry* 2021, 22, 100301.
6. Sigman, M.E.; Williams, M.R. Covariance Mapping in the Analysis of Ignitable Liquids by Gas Chromatography/Mass Spectroscopy. *Analytical Chemistry* 2006, 78, 1713-1718.

### Fire Debris, Headspace, Sensitivity Analysis

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## B89 Gasoline Identification by Pattern Recognition: Facts and Myths

*Reta Newman, MA\*, Pinellas County Forensic Lab, Largo, FL; Brenda Christy, MS, Virginia Department of Forensic Science, Norfolk, VA; Kelsey Winters, MS, Virginia Department of Forensic Science, Norfolk, VA; Michael Gilbert, BS, Pinellas County Forensic Lab, Largo, FL*

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**Learning Overview:** Attendees will learn about the results of a study that included a detailed comparison of chromatographic patterns associated with gasoline and negative sample matrices in fire debris analysis with an emphasis on the facts and myths associated with data interpretation of key diagnostic features.

**Impact Statement:** This presentation will impact the forensic scientific community by addressing inconsistencies in the emphasis placed on specific chromatographic patterns in the identification of gasoline in fire debris samples

The identification of ignitable liquids (ILs) is primarily made using pattern recognition techniques comparing key diagnostic features (KDFs) between forensic unknown samples and known ignitable liquids. When complex matrices, especially those that include pyrolyzed synthetic materials, are present, the interpretation becomes increasingly subjective. This presentation will focus on the KDFs of gasoline, providing the facts and debunking the myths on the importance and uniqueness of specific KDFs in the identification of gasoline based upon analyses of 150 gasoline reference samples (acquired from various geographic locations and tested in multiple laboratories) and the analysis of 56 negative debris samples.

Extracted ion profiling (EIP) is used to emphasize chromatographic patterns and isolate KDFs associated with specific compound types (alkylbenzenes, polynuclear aromatics, indanes, alkanes, cycloalkanes). Historically, greater emphasis has been placed on specific KDFs (for example C3 alkylbenzenes) and less on others in gasoline identification. This presentation will include a comprehensive and objective look at various peak patterns, and in some cases, specific compound identification, used in the forensic community to differentiate gasoline from matrix contributions. Pattern uniqueness was evaluated by comparing closely eluting peak pair ratios in KDF peak patterns.

Ultimately, it was determined that many of the KDFs that analysts currently rely on, were less unique than anticipated, other features including features not commonly reported in literature as being diagnostic, were found to be highly consistent in the reference gasoline samples and less common in matrix samples. The study reiterates the importance of having concordance among many KDFs when differentiating gasoline from complex matrices.

*This work was supported by the National Institutes of Justice grant number 2018-DU-BX-0174.*

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### Fire Debris Analysis, Ignitable Liquids, Pattern Recognition

## B90 Standards Development Activities Related to Ignitable Liquids, Explosives and Gunshot Residue

*Brenda Christy, MS\*, Virginia Department of Forensic Science, Norfolk, VA*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards related to ignitable liquids, explosives (ILE) and gunshot residue (GSR).

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities related to ILE and GSR.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, ignitable liquids, explosives, and gunshot residue standards development activities will be discussed. These include recent standards that have been added to the OSAC Registry:

- ASTM E1388-17 Standard Practice for Static Headspace Sampling of Vapors from Fire Debris Samples
- E1412-19 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal
- E1413-19 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Dynamic Headspace Concentration onto an Adsorbent Tube
- E3189-19 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Static Headspace Concentration onto an Adsorbent Tube
- E3245-20 Standard Guide for the Systematic Approach to the Extraction, Analysis, and Classification of Ignitable Liquids and Ignitable Liquid Residues in Fire Debris Samples – drafting documents to submit for registry approval
- E3197-20 Standard Terminology Relating to Examination of Fire Debris
- E1588-20 Standard Practice for Gunshot Residue Analysis (GSR) by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry (SEM/EDS)

OSAC proposed standards:

- Standard Classification for Ignitable Liquids Encountered in Forensic Fire Debris Analysis
- Standard Test Method for Interpretation of Gas Chromatography-Electron Ionization Mass Spectrometry
- Data for the Identification of Ignitable Liquid Classes in Forensic Fire Debris Analysis
- Standard Guide for Analysis of Ignitable Liquids by Gas Chromatography Electron Ionization Mass Spectrometry
- Standard Practice for the Analysis of Organic Gunshot Residue (oGSR) by Gas Chromatography - Mass Spectrometry
- Standard Practice for the Analysis of oGSR by Liquid Chromatography - Mass Spectrometry
- Standard Practice for the Collection of Primer Gunshot Residue (pGSR) Particles from Clothing, Vehicles, and Other Objects using Adhesive Lifts
- Published standards from the ASTM that have yet to go through the Registry approval process
- Standard Practice for Reporting of Forensic pGSR Analysis by SEM/EDS
- E2451-13 Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples
- Standard Terminology Relating to the Examination of Explosives
- Documents currently in development at the OSAC or by the SDO
- Revision to E1386-15 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent
- WK75150 Standard Practice for Training in the Forensic Examination of Fire Debris
- Standard Guide for the Forensic Analysis of Explosives by PLM,
- Standard Practice for the Characterization of Solid Oxidizer/Fuel Explosive
- WK72856 Standard Practice for Collection and Preservation of oGSR
- WK72526 Standard Practice for Expert Opinions on the Interpretation of pGSR by SEM/EDS
- WK58457 Standard Practice for Training in the Forensic Examination of GSR Using SEM/EDS
- Priorities for new documents or work products
- Fire Debris and Explosives annexes for ASTM E2549
- Standard Practice for Validation and Verification of Analytical Methods for Forensic Science
- Service Providers Performing Forensic Chemistry Analysis
- Standard Guide for the Performance of Automated pGSR Analysis by SEM/EDS
- Standard Guide for Reacquisition of pGSR Particles by SEM/EDS
- Standard Guide for the Classification of pGSR particles by SEM/EDS analysis

### **Ignitable Liquids, Explosives, Gunshot Residue (GSR)**

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## **B91 Standards Development Activities in Firearm and Toolmark Examination**

*Jennifer Floyd BS\*, Arkansas State Crime Lab, Little Rock, AR*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards related to the examination of firearms and toolmarks.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to firearm and toolmarks.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards. The Academy Standards Board (ASB) was formed by the American Academy of Forensic Sciences (AAFS) to accomplish the task of completing and publishing Standards, Best Practice Recommendations, and Technical Notes. To that end, the Firearms and Toolmarks Consensus Body was created.

During this presentation, updates related to firearm and toolmark evidence will be presented. These include i) recent standards that have been added to the OSAC Registry; ii) OSAC proposed standards; iii) published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process; iv) documents currently in development at the OSAC or by the ASB; v) priorities for new documents or work products, and other highlights.

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### **Firearm and Toolmarks, Standards, OSAC**

## **B92 Bullet Ricochet of Polymer Coated Bullets**

*Stephen Daly\**, John Jay College of Criminal Justice, New York City, NY; *Peter Diaczuk, PhD*, John Jay College of Criminal Justice, New York City, NY

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the bullet ricochet of polymer coated bullets off of various substrates.

**Impact Statement:** This presentation will impact the forensic science community by providing a better understanding of the unique characteristics of one brand of polymer coated bullets.

Polymer coated bullets were designed to limit a shooter's exposure to lead as well as to limit the amount of lead that would be released into the environment. However, despite the advantages that these bullets give there are still difficulties which may arise in a forensic firearms examination. Unlike in a typical metal coated bullet, individualizing striations will typically not be imparted to a polymer coated bullet making it unable to be linked to a specific firearm. In fact, the only striations that are typically imparted to the polymer coated bullets are those of the general rifling characteristics which cannot be used to link a bullet to a specific firearm. Despite the difficulties that may arise there is still little known about these polymer coated bullets and there have been few published works regarding these bullets. Due to this difficulty any information that can be gained at the scene of a shooting could be crucial in a forensic firearm examination. Some obtainable information includes the angle of ricochet and the angle of incidence of the discharged bullet as well as traces from the polymer coating found at the impact mark. With this information, details such as the location of the shooter, the angle at which the shot was taken, or the angle at which the bullet ricocheted from the substrate are obtainable. In this project the ricochet angle and angle of incidence, utilizing the ellipse method, were calculated using trigonometric equations. The velocity of the shots was also measured using a chronograph.

The data was collected using 9 mm Luger Federal brand total synthetic jacket (TSJ) bullets and 9 mm Luger Federal brand full metal jacket (FMJ) bullets, which were used as a comparison point. The selected substrates were sheetrock<sup>®</sup>, sheet metal, and durock<sup>®</sup> cement boards. Upon the collection of the velocity data, the TSJ bullets had a significantly lower average velocity (p-value of <0.00001), by a difference of 257 ft/s (feet per second) when compared to the FMJ average velocity. When the angles of ricochet were calculated each FMJ average angle was lower than the corresponding TSJ average angle, except in two instances. The ellipse method was found to still be an accurate method to determine the angle of incidence when applied to impact marks from TSJ bullets. In many of the impact marks there was also a red residue left behind which would have to be from the red polymer coating of the bullets.

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### **Bullet Ricochet, Polymer-Coated Bullets, Syntech Ammunition**

**B93 A Forensic-Related Study on AK Bullet (7.62mm X 39mm) Holes on 1mm Sheet Metal and the Designing of the First Android-Based Field Investigation Tool for AK Gun-Related Bullet Trajectory Reconstructions**

*Bandula Nishshanka, MSc\*, Centre for Defence Research and Development, MOD, Sri Lanka, Colombo, Western, Sri Lanka; Randika Ariyaratna, MSc, Centre for Defence Research and Development, MOD, Sri Lanka, Colombo, Western, Sri Lanka; Chris Shepherd, PhD, University of Kent, Canterbury, United Kingdom*

**Learning Overview:** The presentation introduces a new method to identify the trajectories of AK bullets using the size of the bullet hole on 1 mm sheet metal and automotive sheet metal. The participant can learn the new theory and practically employ the theory and the new android tool for future AK gun related vehicle shooting incidents.

**Impact Statement:** The presentation introduces a new method and an android based field investigation tool to identify the trajectories of fired AK bullets using the size of the AK bullet hole on automotive sheet metal. This is a new method with proved success in field trials and wishes to introduce it to the shooting investigators worldwide. This new method can be used for AK investigations worldwide and introduce a novel method to the existing methods for trajectory determination of bullets from bullet holes. As AK guns have become a threat to the world peace with the increase reporting in recent crime scenes worldwide, this study will have a considerable impact to the field of AK gun related shooting investigations.

Bullet holes play a major role in the trajectory determination of fired bullets, potentially suggesting where the shots were fired from. Few methods are currently employed to estimate the angles of incidence of fired bullets using bullet holes. The study presents the findings of an empirical study and the design of the first android-based field investigation tool for AK gun-related vehicle shooting incidents, based on the relationship identified with the angle of incidence and length of the bullet hole in automotive sheet metal.

In the field experiment, AK bullets (7,62 mm x 39 mm) were fired into 1 mm sheet metal placed at different angles from 90 to 15-degrees and the results demonstrated a strong inverse relationship (Figure 1). This relationship has not been used as a method to estimate the angle of incidences of fired bullets and the findings confirmed that, the lengths of the bullet holes *l* can be used as a viable indicator to estimate the angle of incidence of fired bullets to vehicle bodies in real crime scenes. The full paper published in this study is available at AAFS Journal; *Journal of Forensic Sciences*; <https://onlinelibrary.wiley.com/doi/abs/10.1111/1556-4029.14717><sup>1</sup>

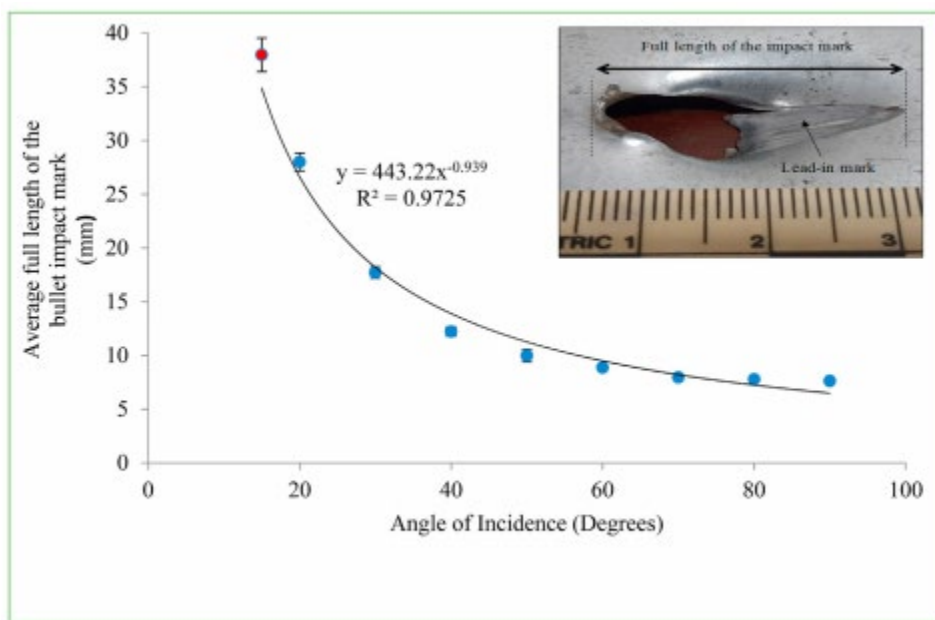


Fig 1. The relationship identified between the angle of incidences of Ak bullets and the length of the bullet hole on 1 mm sheet metal.

Continuing the study and utilizing the study’s findings to a greater particle use, the team developed a mobile-based software tool, based on the formula identified on the correlation of the incident angle of AK bullets and length of the bullet hole. The designed software tool: “Bullet Trajectory Plotter” could estimate the potential trajectories of perforated AK bullets in 1 mm sheet metal surfaces. The software tool proved to be a viable, quickly employable, user-friendly, and fully mobile field investigation tool that can be installed on investigators’ mobile phones to identify the approximate angles of incidence of perforated AK bullets on 1 mm sheet metal surfaces.

The tool was field-tested using a van door with a 1 mm sheet metal body. The new tool and the commonly used ellipse method were employed to estimate the angles of incidence of fired AK bullets, and the estimated values were recorded. The results of the estimated trajectory from the new tool and the comparison of the mean differences reported in the two methods compared to the real angles of incidence of fired AK bullets are illustrated in Fig. 2.



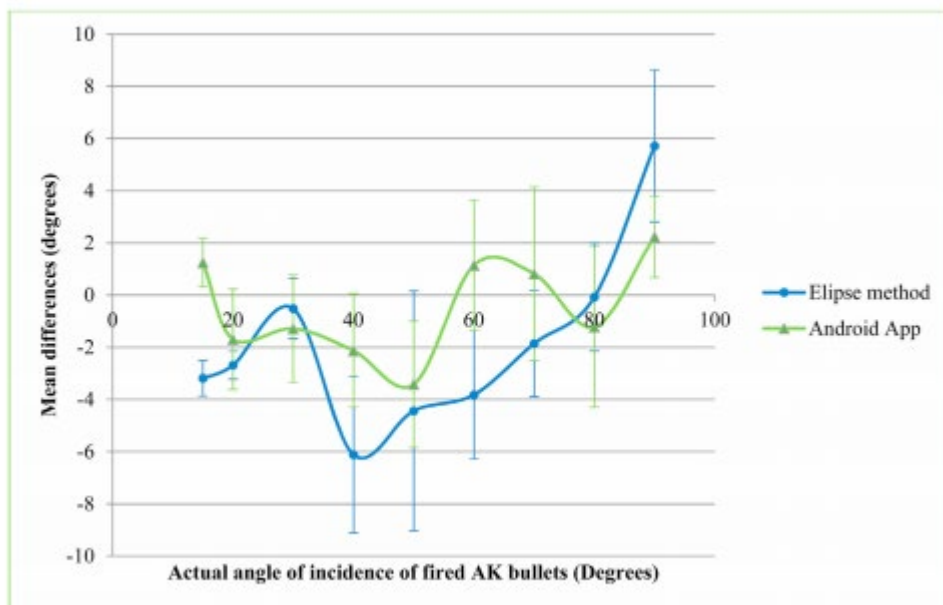


Fig 2: A comparison of the mean differences reported in the estimated angles of incidence, using two methods compared to the real angles of incidence of fired AK bullets.

The results of the field tests highlighted the employment of the new method in AK-related investigations. It is also significant to highlight that, the estimated incident angles by the new software tool were within  $\pm 5$  - degrees universally accepted error margin to the actual incident angles in bullet trajectory reconstructions. Some of the screen captured image of the developed software is given at Fig 3 and the full content of the published paper in this regard is available at: <https://www.sciencedirect.com/science/article/abs/pii/S2666281721001840>.<sup>2</sup>

**Reference(s):**

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2. <https://www.sciencedirect.com/science/article/abs/pii/S2666281721001840?dgcid=author>

**AK Guns, Shooting Reconstruction, Android™ Tool**

## B94 Forensic Discrimination of Aluminum Sources in IEDs using Quantitative Trace Elemental Analysis

*Anjali Bhandari, PhD\*, ORISE/FBI Laboratory, Quantico, VA; Michelle Jordan, PhD, Carnegie Institute for Science, Washington, DC; Christopher Saunders, PhD, South Dakota State University, Brookings, SD; Danica Ommen, PhD, Iowa State University, Ames, IA; JoAnn Buscaglia, PhD; FBI Laboratory, Quantico, VA*

**Learning Overview:** Attendees of this presentation will gain a comprehensive overview of trace elemental profiles via ICP-MS from a variety of aluminum-based sources, understanding the ability of the developed method to discriminate between sources.

**Impact Statement:** Research presented will help the community understand the value in using ICP-MS to quantitate trace element compositions for discriminating between aluminum-based sources to further characterize IED component sources.

Improvised explosive devices (IEDs) often contain inexpensive, easily accessible materials. Metallic aluminum (Al) is commonly used as the fuel base in IEDs to increase the heat of reaction. Al powders can be produced from commercially available Al sources including foils, pastes, pyrotechnics, spray paints, Al cans and components of binary exploding targets. At AAFS 2020, method development and proof-of-concept research was presented<sup>1</sup>. Further method refinements as well as new data evaluating the discrimination of Al sources using trace element concentrations by inductively coupled plasma mass spectrometry (ICP-MS) will be presented.

Koons, et al.<sup>2</sup> initially investigated ten trace element concentrations in Al foils in 1993 and demonstrated that trace elemental profiles allowed for discrimination between the 12 manufacturers studied, as well as between two lots of the same manufacturer for the 30 rolls analyzed. Research presented here builds on these initial findings and analyzes a larger, more diverse sample set using instrumentation with greater sensitivity, which permits analysis of additional elements.

The current Al source collection contains approximately 381 sources from different starting materials and final manufactured powders, a variety of global manufacturers, as well as materials from both the same and different production lots of the same manufacturer.

Twenty-nine elements of interest are quantitated via ICP-MS using two states: standard (STD) and kinetic energy discrimination (KED) depending on the isotope and potential interferences. To ensure and verify accuracy and stability, external calibrations are used, a multielement internal standard covering the analyzed mass range is added to all samples to correct for instrumental drift, and a NIST-certified standard reference material is analyzed with all samples.

Previously reported homogeneity tests<sup>1</sup> were conducted using a 250-foot Al roll sampled every 10 feet. Based on these results, it was concluded that the roll was likely not significantly heterogenous and that the trace element profile was independent of the sampling location. Preliminary tests also confirmed that trace element profiles were distinct among two different manufacturers, allowing for between-source discrimination. Based on these initial findings, a statistical subsampling scheme was implemented using nine locations along the length of each Al foil roll. Specific subsamples of paired sources were analyzed over the course of two-day analytical runs in a designated pattern. Forty-six sources from 39 distinct manufacturers were analyzed in this manner. Statistical analysis failed to detect significant within-roll heterogeneity; therefore, subsequent sampling was reduced to the first 18 inches of the roll using the same sampling scheme.

Trace element concentration data from Al sources were analyzed statistically, assessing points of variability in both the analytical measurement and within/between Al sources. Statistical analysis methods are used to determine which specific analytes or suite of analytes, if any, offer the greatest discrimination between sources from different manufacturers or products/lots of the same manufacturer. Information learned from analysis of this sample collection will assist in providing further characterization of Al powder sources used in IEDs and may provide valuable lead information for investigations and intelligence.

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### Trace Elements, Aluminum, ICP/MS

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**B95 Loading Artificial Fingerprints onto Brass Shell Casings for Improved Method Development and Proficiency Testing**

*Curt Hewitt\*, Signature Science, Austin, TX; Danielle LeSassier, Signature Science, Austin, TX; Benjamin Ludolph, Signature Science, Austin, TX; Kathleen Schulte, Signature Science, Austin, TX; Myles Gardner, Signature Science, Austin, TX*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of challenges of DNA analysis using brass shell casing samples and emerging methods to improve and standardize DNA analysis from this challenging matrix.

**Impact Statement:** This presentation will impact the forensic science community by presenting novel approaches to generate standardized brass shell casing samples for DNA collection method development or for potential use in proficiency testing shell casing extraction methods.

No two human touch samples are the same. This variability complicates research focused on DNA collection and extraction methods. To this end, artificial fingerprints containing precise amounts of DNA and sebaceous material have been utilized to prepare highly standardized samples for method development purposes.<sup>1</sup> However, these approaches are not extensible to one of the most challenging forensic matrices: brass shell casings. While heat and pressure play a role in DNA degradation on shell casings, chemical damage by reactive oxygen species (ROS) containing copper molecules has perhaps a greater effect on stability. This is illustrated by high rates of DNA degradation observed from unfired brass shell casings and on other copper surfaces that exhibit similar properties. These limitations lead to exaggerated DNA degradation in artificial fingerprint samples when compared to human donor samples, likely due to the contrived environment caused by the addition of naked genomic DNA to the metal surface in an aqueous solution.

To assess whether the formulation of artificial fingerprints could be modified and optimized for use on brass shell casing samples, various additives and workflow alterations were evaluated. Creating physical separation between the DNA and shell casing surface by first applying a layer of synthetic sebaceous material was not successful. Depositing a hydrophobic layer onto the shell casing did reduce DNA degradation but created an environment that was overly contrived.

Next, various additives, such as chelators, were evaluated to determine whether they could protect the DNA during the artificial fingerprint drying process. EDTA increased degradation as observed by green discoloration on the shell casing surface over time. Other protein additives, such as BSA or a tripeptide mixture previously reported to aid in DNA recovery from shell casings, also failed to prevent excessive DNA degradation.<sup>2</sup>

Finally, rapid desiccation and storage methods were assessed with the aim of limiting the amount of time that solubilized ROS molecules could come into contact with hDNA. By placing shell casings containing artificial fingerprints directly into a SpeedVac following preparation, DNA stability increased 50-90% over a 48-hour time period. Degradation Index (DI) values using a commercial DNA quantification kit also decreased to < 5, as opposed to DI values > 10 for non-dried samples. Storage under desiccation further extended DNA stability, suggesting that moisture from the air can act to rehydrate a sample over time.

Taken together, our findings establish a method for the deposition of artificial fingerprints on brass shell casings that mimic human deposits. Critically, by rapidly drying the artificial fingerprint samples, it is possible to avoid excessive degradation caused by prolonged exposure of naked genomic DNA in an aqueous environment to the copper and other reactive molecules on the shell casing surface. This data may also inform shell casing forensic analysis, suggesting that samples should be stored under desiccating conditions to reduce exposure to moisture and damaged caused by ROS.

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**Brass Shell Casings, DNA Analysis, Artificial Fingerprints**

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## **B96 Standards Development Activities Related to the Examination of Friction Ridge Detail**

*Heidi Eldridge, PhD\*, RTI International, Research Triangle Park, NC*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards related to the examination of friction ridge detail from the hands and feet.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to the examination of friction ridge detail.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, updates related to friction ridge standards development activities will be presented. These include i) recent standards that have been added to the OSAC Registry; ii) OSAC proposed standards that are currently published on the OSAC website in draft form; iii) published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process; iv) documents currently in development at the OSAC or by the ASB; and v) priorities for new documents or work products. This presentation will also highlight how the process of moving documents back and forth between the OSAC and ASB works for the friction ridge discipline, challenges and successes that process has encountered, and what the near future looks like for the promulgation of friction ridge standards and guidelines.

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### **Friction Ridge, Standards, OSAC**

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## B97 Understanding and Generating Close Non-Matches in Latent Prints for Training, Testing, and Research

*Heidi Eldridge, PhD\*, RTI International, Research Triangle Park, NC; Christophe Champod, PhD, University of Lausanne, Lausanne, Vaud, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will be aware of the creation of an International Close Non-Match Library (ICNML) for latent prints and the results of testing that was done to validate expert consensus-based criteria for predicting the marks that are more likely to produce close non-matches.

**Impact Statement:** This presentation will impact the forensic science community by informing them of a new resource that can be used to create known ground truth exercises for training, testing, and research that will include challenging close non-match pairings. It will also present the results of research to validate criteria used to predict those marks that are likely to produce close non-matches, which will aid latent print examiners in training to avoid false identifications due to close non-matches.

Close non-matches (CNMs) (marks that look similar but originated from different sources) present a challenge for examiners because they are the most likely to lead to false-positive errors. As the size of Automated Fingerprint Identification Systems (AFIS) increases, the chance of encountering a CNM in casework also increases. Yet there is little training available in avoiding these errors because there are few known cases available to study.

The authors have leveraged an international team of collaborators to create the first known International Close Non-Match Library (ICNML), which is populated with known ground truth same source, different sources, and CNM pairings. This database will be made available free of charge to trusted law enforcement partners for testing, training, and research purposes. Access to such a database will allow for challenging exercises to be built that will help train examiners to avoid making false identification errors due to CNMs.

The CNMs collected in this study were found by searching clusters of minutiae through multiple AFIS according to selection criteria developed by an expert panel of latent print examiners with background and expertise in CNMs. Once the database was populated with CNM pairs, research was conducted to validate these criteria by testing whether different sources pairings located using the criteria resulted in higher false positive error rates than different sources pairings that were found through AFIS searches on random configurations of minutiae (thus demonstrating that more examiners were misled into false identifications by the pairings found through targeted searches according to CNM criteria). Results of this research will be presented along with a presentation of the developed criteria and whether they should be incorporated into training on CNMs. Finally, attendees will be informed of how to access the ICNML to download comparison packets for use in their own agencies.

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### Latent Prints, Close Non-Match, AFIS

## B98 How Do Latent Print Examiners Perceive Blind Proficiency Testing? A Survey of Practicing Examiners

Brett Gardner, PhD\*, University of Virginia, Charlottesville, VA; Sharon Kelley, PhD, University of Virginia, Charlottesville, VA; Maddisen Neuman, MA, Houston Forensic Science Center/CSAFE, Houston, TX

**Learning Overview:** This program will educate attendees about preliminary findings from a survey administered to practicing latent print examiners. This presentation will describe beliefs regarding blind proficiency testing commonly held by examiners, explore how beliefs differ between examiners who do and do not work for a laboratory with such procedures, and detail the importance of understanding examiner perceptions when attempting to implement blind proficiency testing.

**Impact Statement:** This presentation will describe examiner perceptions of blind proficiency testing and identify differences among examiners who currently participate in such testing. Audience members will be encouraged to reflect upon their own perceptions of blind proficiency testing and discuss how concerns raised by examiners can inform attempts to implement blind testing procedures in laboratories.

**Introduction:** In the wake of criticism regarding traditional proficiency testing, there has been an increased focus on implementing blind proficiency testing (BPT) in crime laboratories.<sup>1-4</sup> Indeed, landmark reports have strongly recommended the pursuit of BPT in crime laboratories.<sup>5,6</sup> To date, the implementation of BPT in latent print units has remained limited.<sup>7</sup> Scholars have reviewed obstacles for implementation, and one identified challenge has been the “cultural history in the field,” i.e., examiner preconceptions about proficiency testing.<sup>8</sup>

The current study sought to explore perceptions of BPT among practicing latent print examiners and determine whether such beliefs varied between examiners who work for laboratories with and without BPT.

**Method:** Researchers administered an online survey to practicing latent print examiners about their typical work practices and perceptions of BPT. Specifically, examiners indicated their opinion of the value of BPT beyond non-blind proficiency testing in accurately assessing examiner proficiency, on a scale of 0 = *Completely Unnecessary* to 10 = *Absolutely Necessary*. They also indicated their agreement with statements that BPT: (1) is a valuable use of time/resources, (2) increases examiner diligence, (3) improves testimony experiences, and (4) reveals laboratory areas most vulnerable to errors. Finally, examiners indicated their agreement with statements suggesting that they are/would be glad to work in a laboratory with BPT, and that examiners in their laboratory feel positively about BPT. The final sample included 338 latent print examiners. Although most examiners practiced within the United States (88.5%), participating examiners practiced in at least 11 other countries.

**Results:** Overall, opinions regarding the value of BPT to accurately assess examiner proficiency varied widely. On a scale from 0 to 10, some examiners described BPT as “completely unnecessary” and others described BPT as “absolutely necessary,” with most examiners considering such testing to be somewhat necessary ( $M = 6.3$ ).

The presentation will provide detailed results describing examiner beliefs. In brief, examiners “somewhat disagreed” with the notion that BPT would increase examiner diligence but endorsed ambivalent beliefs about other aspects of BPT. On average, examiners slightly disagreed with the notion that BPT would improve their testimony experience, and that examiners feel positively about BPT. Examiners slightly agreed that BPT is a valuable use of time/resources and that they are/would be happy to work in a laboratory with such procedures.

Approximately 14.8% of examiners indicated that their laboratory implements BPT in latent print comparison. Examiners who work within a laboratory with BPT endorsed more positive beliefs about all aspects of blind testing. As a primary example, examiners who work in a laboratory with BPT view such testing as significantly more valuable in accurately assessing examiner performance than other examiners,  $t(269) = 5.93, p < .001, d = 1.04$ .

Results will also describe the benefits and downsides of BPT. In brief, examiners often indicated that BPT would reduce bias in testing procedures, provide more representative and accurate assessment, and enhance court testimony. On the other hand, many examiners indicated that BPT is logistically challenging and consumes limited time and resources. Results will present differences in identified benefits and downsides among examiners who do and do not work in a laboratory with BPT.

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### Blind Proficiency Testing, Examiner Perceptions, Latent Prints

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## B99 What Types of Information Can and Do Latent Print Examiners Review? A Survey of Practicing Examiners

Brett Gardner, PhD\*, University of Virginia, Charlottesville, VA; Sharon Kelley, PhD, University of Virginia, Charlottesville, VA; Maddisen Neuman, MA, Houston Forensic Science Center/CSAFE, Houston, TX

**Learning Overview:** This program will educate attendees about findings from a survey administered to practicing latent print examiners. This presentation will identify what types of information examiners can access during routine work, and what types of information examiners typically choose to review. Results will describe how patterns in information access and review vary by unit size and examiner roles within the laboratory.

**Impact Statement:** This presentation will describe common examiner work practices in routine latent print comparisons regarding what information is typically accessed and reviewed. Audience members will be encouraged to reflect upon their own work practices and contemplate what types of information should and should not be reviewed while completing latent print comparisons.

**Introduction:** Understanding typical work practice is important to understanding latent print comparison and ultimately improving the reliability of the discipline. ACE-V procedures provide general structure, but research suggests that adherence to ACE-V standards is unknown and examiner decisions continue to vary within every phase of work procedures.<sup>1-3</sup>

Reducing exposure to task-irrelevant information is one way to reduce bias and improve inter-examiner reliability in latent print comparison.<sup>4,5</sup> But research shows that examiners disagree regarding what information is task-relevant.<sup>6</sup> Further, some examiners have indicated that implementing large-scale procedures (such as linear sequential unmasking) is unfeasible in small laboratories due to personnel and resource restrictions.<sup>7</sup> For example, examiners in small laboratories sometimes must fulfill administrative roles in addition to their analytical duties. Expanding upon the limited task-relevance literature, the current study surveyed practicing latent print examiners about their routine work practices. This study specifically sought to review what types of information examiners typically review when conducting their analyses and explore whether access and inclination to review different information vary according to examiner role (i.e., supervisory/managerial examiner vs. nonsupervisory examiner) and unit size.

**Method:** Researchers administered an online survey to practicing latent print examiners. The survey requested information regarding examiners' current position, laboratory details, and typical work practices. Specifically, examiners indicated which types of information (of 14 listed information types; e.g., suspect age, victim name) they had access to in their daily work, and which types of information they routinely reviewed. The final sample included 284 latent print examiners that provided complete responses. Of these, most examiners practiced within the United States (91.5%), although participating examiners practiced in at least 10 other countries.

**Results:** The final presentation will provide detailed demographic information about surveyed examiners and their laboratories. Although results are only briefly summarized here, the presentation will present comprehensive information and tables about all analyses. In short, results revealed significant variability in the types of information to which examiners have access and review. Most examiners (i.e., ~75% or higher) have access to information regarding evidence description, method of evidence collection, offense type, suspect name, and victim name. However, only evidence description and method of evidence collection are routinely reviewed by most (i.e., >50%) examiners. In fact, approximately half of examiners (51.4%) indicated that they did not review offense type information despite having access to such information.

Differential patterns in accessing and reviewing information types also emerged according to the size of an examiner's latent print unit and their role within the laboratory. These patterns will be detailed and discussed. In brief, examiners from small latent print units (i.e., ≤3 examiners) were more likely to have access to every type of information listed, and they more often indicated that they routinely reviewed 11 of 14 information types compared to examiners from large units (i.e., ≥10 examiners). However, examiners from smaller units were also more likely to indicate that they actively did not review 11 of 14 accessible types of information compared to examiners from larger units. Thus, results indicate that examiners in larger units do not review information as often as those from smaller units because they do not have access; examiners from smaller units must actively decide to not review available information (and often do). Examiners in supervisory/managerial roles differed from other examiners in that they were less likely to review all information types compared to other examiners. Implications for practice and laboratory operating procedures will be discussed.

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1. Langenburg, G.M. Pilot study: A statistical analysis of the ACE-V methodology – Analysis stage. *Journal of Forensic Identification* 54, (2004): 64-79.
2. Ulery, B.T.; R. Hicklin; G. Kiebuszinski; M. Roberts; J. Buscaglia. Understanding the sufficiency of information for latent fingerprint value determinations. *Forensic Science International* 230, (2013): 99-106.
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### Latent Print Comparison, Task Relevance, Contextual Effects

## **B100 Does Image Editing Improve the Quality of Latent Prints? An Analysis of Image-Enhancement Techniques in One Crime Laboratory**

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**Learning Overview:** This program will educate attendees about findings from a study examining the prevalence of image-editing during routine casework in the Latent Prints section at the Houston Forensic Science Center. Additionally, this presentation will detail the impact of image-editing techniques upon the quality and clarity of latent prints, and explore how this information can be used to improve and standardize laboratory procedures.

**Impact Statement:** This presentation will evaluate typical image-editing procedures within one latent print unit and examine the impact of such procedures on print quality and clarity. Audience members will be encouraged to reflect upon their practice and any guidance that their laboratory may provide on image-editing given the observed variability in routine practice.

**Introduction:** In the wake of calls for reform and additional empirical research in many forensic science disciplines, the literature describing latent print comparison has significantly increased in recent years.<sup>1,2</sup> Indeed, scholars have examined basic cognitive processes involved in latent print comparisons (e.g., eye tracking studies and contextual bias studies), examiner reporting practices, and juror perceptions of resulting latent print testimony.<sup>4,7</sup> However, most of the extant latent print research relies on highly controlled experimental designs that are not always representative of actual casework. Field studies, or studies assessing examiner behavior during the course of actual casework, are necessary to increase understanding of basic processes associated with latent print comparison.

One recent field study revealed that print quality (as measured by objective quality metrics) is associated with sufficiency determinations, examiner conclusions, and examiner accuracy.<sup>8</sup> “Good” quality latent prints were more than twice as likely to result in correct conclusions as were “Ugly” quality prints.

The current study addressed an important, but under-studied aspect of the latent print comparison process: the practice of digitally editing latent print images. Anecdotally, examiners often alter the images of latent prints, but image-editing behavior is inconsistent among examiners. There are no standards mandating or restricting the editing of latent print images. This study therefore sought to: (1) provide information on the prevalence of latent print image editing within one laboratory (i.e., Houston Forensic Science Center; HFSC) and (2) examine the potential effect of image editing on objective print quality and clarity, as well as analyze potential examiner differences in editing effectiveness.

**Method:** This study reviewed digital images of 378 latent prints analyzed by examiners as part of 144 blind cases. Researchers obtained images of latent prints that had been edited and uploaded into the case management system and retrieved original copies of the edited images to obtain unedited images of the same prints. Both the original and edited images of each print were then scored using the FBI’s Latent Quality Metrics (LQMetrics) software to obtain objective measures of print clarity and quality.<sup>9</sup>

**Results:** The vast majority of collected latent print images (98.9%) had been edited in some fashion as part of routine casework. Excluding cropping and rotation edits, 91.0% of images had been enhanced in some manner. A review of the editing histories suggested that common editing techniques included: Tonal Reversal, Brightness/Contrast Adjustment, Color Alteration, and Shadow and Highlight Adjustment.

A paired-sample *t* test revealed that, on average, edited latent print images ( $M = 53.4$ ) were of slightly higher quality than raw latent print images ( $M = 50.8$ ),  $t(371) = 4.77$ ,  $p < .001$ ,  $d = .13$ . Although edited images were only slightly higher quality than raw images, another paired-sample *t* test revealed that, on average, edited latent print images ( $M = 34.3$ ) were of meaningfully higher clarity than raw latent print images ( $M = 27.6$ ),  $t(373) = 19.96$ ,  $p < .001$ ,  $d = .55$ .

Additional results indicated that examiners did not significantly vary in their ability to increase the quality of print images but did vary significantly in their ability to increase the clarity of edited print images. The presentation will provide detailed analyses and tables describing examiner differences. Ultimately, findings identify a source of variability in examiner behavior and emphasize a need for laboratory guidance regarding image-editing procedures.

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

1. National Academy of Sciences. *Strengthening Forensic Science in the United States: A Path Forward*. (2009). Washington, DC: National Academies Press.
2. President’s Council of Advisors on Science and Technology. *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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### **Latent Print Comparison, Image Editing, Quality Metrics**

## **B101 Validation of a Systematic Method for Duct Tape Physical Fits Through Inter-Laboratory Studies**

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**Learning Overview:** After attending this presentation, attendees will learn about the results of two inter-laboratory studies designed to validate a developed systematic method for reporting the quality of a physical fit of duct tape edges.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the value of developing systematic methods for conducting physical fits in trace evidence. This presentation will also demonstrate the utility of the developed method for duct tape and highlight how the documentation of critical features and a quantitative similarity score can offer additional support to inform the examiners' expert opinion.

Physical fit examinations involve analyzing separated items to determine if the compared edges re-align with distinctive features. In trace evidence, physical fits are considered the highest degree of association between two items of evidence. Currently, there is an effort by the OSAC Trace Subcommittee to develop consensus-based criteria and methods to provide a more standard approach to physical fit examinations. Previously, our group developed a systematic method for physical fits of duct tapes, utilizing an edge similarity score as a quantitative metric of the quality of a fit to support the examiner's opinion.<sup>1</sup> In this study, the developed method was evaluated by forensic practitioners under two interlaboratory studies. In both studies, a set of seven duct tape pairs were provided to each participant. Before distribution to participants, the samples were examined by an expert panel to determine a baseline consensus similarity score. The tests were designed as a Round Robin, including fracture edges of true fits and true non-fits of different difficulty levels to resemble casework items. Participants were asked to provide their opinion on a potential fit between each pair of tape edges, as well as report the respective edge similarity score. The feedback received from the first study was applied to refine the instructions, training, and reporting tools. As a result, the second interlaboratory study added more focus to the documentation of the type and location of critical features each examiner used to make their decision and added an option of reporting inconclusive on each comparison sub-unit. The reporting template was simplified with autofill cells, automatic calculations of the ESS, and a code that automatically colored a fit, non-fit or inconclusive cell with green, red, or yellow, respectively, for ease of visualization of the aligning patterns. Features such as scrim pattern weave, aligning warp and weft fibers, severed dimples on the backing, and distortion of the backing and adhesive were documented at each pair's macroscopic and microscopic levels. Also, participants were required to participate in an instructional training session before taking the test. The video was also made available for review at any time. The participants generally reported the overall determination of fit or non-fit for each pair accurately and were able to document which features contributed to that decision at each comparison step. A high level of agreement between participants and the consensus ESS values was observed, demonstrating the utility of the comparison approach. The performance rates showed no false positives and low false negatives (<5%), with scores above 80 providing strong support for a fit and scores below 30 providing strong support for non-fits. Overall, the two interlaboratory studies demonstrated that the proposed comparison method provides examiners with support for their decisions with consistent documentation of observed features and a quantitative score to help describe the quality of the fit.

Moreover, the approach can be leveraged to provide different measures of the evidentiary value of fracture matches in duct tape evidence, as demonstrated in a previous population study of over 3000 duct tape comparisons using score likelihood ratios.<sup>1</sup> One of the main benefits of the ESS method is that it is systematic, facilitating standardization of comparison criteria and data-driven conclusions. An added value of this approach is its ability to transparently demonstrate the thought process in peer-review and as a training tool. And finally, because qualitative and quantitative descriptors are documented, it helps not only in the standardization of protocols but also in the demonstration of scientific validity that can serve as a foundation for supporting the value of physical fits and a better understanding of the factors that can influence accuracy.

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

- <sup>1</sup>. Prusinowski, M.; Brooks, E.; Trejos, T. Development and Validation of a systematic approach for the quantitative assessment of the quality of duct tape physical fits. *Forensic Science International*. 2020; 307: 110103

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### **Physical Fits, Interlaboratory Studies, Systematic Comparison**

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## **B102 Student Perceptions of the Forensic Science Major at Middle Tennessee State University**

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**Learning Overview:** After attending this poster presentation, viewers will have a greater understanding of currently enrolled Middle Tennessee State University Forensic Science Program students' perceptions of program performance and curriculum collected from both quantitative and qualitative survey questions.

**Impact Statement:** This presentation will impact the forensic science community by sharing the responses of currently enrolled students in the MTSU forensic science program, granting faculty members insight on the drawbacks and benefits of current program mechanisms and how to influence them for the better.

This work was undertaken to better understand what the currently enrolled students in Middle Tennessee State University's (MTSU's) Forensic Science Program desire in their curriculum and their opinions on how to strengthen the program. In the Spring 2021 semester, a total of 46 students out of 180 students currently registered in the program (-25.5% response rate) took a survey created by the authors, designed to address these questions. The survey was created through a survey instrument named Qualtrics. Survey questions debriefed participants beginning with demographical questions, including such questions as enrollment status, age, employment status, gender, and current class qualification. Furthermore, the survey also collected qualitative data with opportunity for survey participants to elaborate. After Qualtrics reported the survey data received from the participants, the authors interpreted the results within Excel documents.

For quantitative data, the survey participants responded to questions among topics such as preference of field work versus lab work in forensics, perception of level of support from faculty advisors as well as from the advising department for the program, and their preferences on mentoring for currently enrolled students. Moreover, they were asked their likelihood to participate in extracurricular activities, whether MTSU was the participants first choice in school, student comfort level in interactions with professors, among other questions. Qualitative survey questions addressed items such as student interest in potential new forensic-based upper-division electives and perceptions of whether the program met their expectations with the opportunity to elaborate.

Overall, the majority of students who took the survey found the program challenging and felt that it properly prepared them for their post-graduation careers. However, the results indicated that the program has not met all the students' expectations, nor have students gained all of the experiences they anticipated. Perceptions of currently enrolled students are provided in this report from an even array of freshman through seniors. A theme discovered from responses regarding expectations of the program was the lack of forensic significance among some coursework in the program, which was unexpected by participants. An expectation met was the balance provided between science and criminal justice classes within the program. Detailed feedback was received about the forensic-based upper-division electives the students desired for the program, with twenty-two potential options provided in the survey. "Death Investigation", "Bloodstain Pattern Analysis", and "Forensic Fingerprinting" were the three most common interests among survey participants with the initial ranking the highest with twenty-seven interested participants. Understanding the perceptions of currently enrolled students may shed new light on the program for the forensic science faculty at MTSU which will allow them to heighten the academic experiences of the students participating in the program. Moreover, the students' thoughts on the benefits of the program give just as much insight as the drawbacks, and all should be considered by the MTSU forensic science faculty.

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### **Forensic Science, Education, Perceptions**

## **B103 Optimization Parameters of Fragmentary Voltage and Collision Energy for Identification and Separation of RDX (1,3,5-Trinitro-1,3,5-Triazinane) From Other Explosives with Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

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**Learning Overview:** After participating in this presentation, participants will present with the Kosovo Agency for Forensic achievements the development and optimization of the parameters for the creation of a new method for identification and separation of organic explosives.

**Impact Statement:** This presentation will affect the scientific community of forensics by describing the current capabilities of the Kosovo legal science lab in the development of new methods for the identification and separation of organic explosives by chromatographic methods and the impact on the identification of source (s) explosives and interconnections to identify terrorists.

Explosives are classified in several ways, based on different criteria. Thus, the explosives are divided into high and low explosives based on the type and speed of the action they are doing. Explosives are also classified according to their chemical structure. The most important group is that of organic compounds containing the nitro (NO<sub>2</sub>) group. They are further subdivided based on the site where NO<sub>2</sub> is attached to the atomic structure. Nitrogen compounds contain C-NO<sub>2</sub> groups, a group of C-O-NO<sub>2</sub> nitrate ethers and C-N-NO<sub>2</sub> nitrite nitriles.

There are different methods for identifying and separating organic explosives, especially RDX (1,3,5-Trinitro-1,3,5-triazinane), but recently, double-mass spectrometric detector chromatography has begun to accommodate numerous scientific research by forensic scientists to find appropriate parameters for the identification and separation of organic explosives in low concentrations in pg (pictograms) or even in fg (fentogram).

Therefore, considering the needs reasonable we have begun to create optimal parameters for CE (collision energy) and FV (fragmentary voltage) for identifying and dividing RDX from other explosives by using the Liquid chromatography instrument with double-mass spectrometric detector (LC-MS/MS) with the Atmospheric pressure chemical ionization (APCI) model, Zorbox SB - C18 column 600 bar 3 x 50 mm 3.5 μm and mobile phase: Methanol / Isopropanol / Water (1: 3: 6) as well as 0.1% chloroform.

In our studies we have found optimal "CE" and "FV" values for the identification and separation of RDX from other explosives, the change of fractional energy values greatly influences the fragmentation of the molecule we are studying, the fragmentation fractions molecule at all levels and this facilitates the identification of the molecule of unknown explosive and all fractions comply with our preliminary knowledge of the RDX structure and knowledge of its potential fragmentation, in addition to the excellent division achieved with this technique and the limit of detection up to fg.

At the end of this study, optimized parameters for identification and separation of organic explosives with LC-MS/MS we have the following conclusions:

- In our study the samples analyzed were organic explosives: HMTD (3,4,8,9,12,13-Hexaoxa-1,6-diazabicyclo[4.4.4]tetradecane), HMX (1,3,5,7-Tetranitro-1,3,5,7-tetrazocane), RDX, TATB (1,3,5-triamino-2,4,6-trinitrobenzene), EGDN (1,2-dinitroxyethane), 1,3,5-TNB (1,3,5-Trinitrobenzene), 1,3-DNB (1,3-Dinitrobenzene), TETRYL (*N*-Methyl-*N*-(2,4,6-trinitrophenyl)nitramide), 4A-DNT (4-amino-2,6-dinitrotoluene), NB (Nitrobenzene), NG (Nitroglycerin), 2A-DNT (2-amino-2,6-dinitrotoluene), TNT (2,4,6-Trinitrotoluene), 2,6-DNT (2,6-Dinitrotoluene), 2,4-DNT (2,4-Dinitrotoluene), 1,3,5-Trinitro-2-[2-(2,4,6-trinitrophenyl)ethenyl]benzene (HNS), 2-Nitrotolueni (2-NT), 4-Nitrotolueni (4-NT), PETN (2,2-Bis[(nitrooxy)methyl]propane-1,3-diyl dinitrate), 3-NT (3-Nitrotolueni), TATP (3,3-Dimethyl-1,2-dioxacyclopropane) and CARBAMITE, creation of methods for identification and separation of explosives doing the optimization of parameters on LC and MS/MS.
- Parameter optimization for both methods done as follow: flow of mobile phase 0.5 ml/minute, temperature of colon 35<sup>o</sup>C and the length of colon 3x50 mm, diameter 3.5 μm, ionization source multimode (MMI), ionization model (APCI), volume of sample injected 20 μm. Mobile phase was suitable for both above methods.

### **RDX, Collision Energy, Fragmentary Voltage**

## **B104 Automated Particle Micromorphometry and Statistical Scoring for Improved Characterization of Aluminum (Al) Powders in Improvised Explosive Devices (IEDs)**

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**Learning Overview:** Attendees will better understand the various statistical methods currently being applied for the interpretation and discrimination of aluminum particles, commonly associated with improvised explosive devices (IEDs). Further, attendees will have a deeper understanding of how automated imaging with measurement of morphological features may aid in forensic science investigations.

**Impact Statement:** This presentation will impact the forensic science community by providing an objective, statistically sound method for the identification of source of aluminum powder evidence in IEDs, providing crucial information for forensic science investigations.

Improvised explosive devices (IEDs) are often composed of commercial or readily available materials. One such material is aluminum (Al) powder; a common metallic fuel, Al powder has many legitimate uses and applications, making it easily accessible from a variety of sources. Although many of these sources are marketed in powder form, other types of sources can be easily and inexpensively manipulated and turned into powder via simple methods found online. This increased circulation of amateur methodologies, as well as their ease of availability, has put dangerous and destructive tools in the hands of amateur bomb makers. For this reason, it has become increasingly important to further characterize Al powder sources beyond identification, in order to differentiate between sources of Al powder for additional investigative and intelligence value.

Building upon the work that has been previously presented at AAFS, it has become increasingly evident that additional discrimination of Al powder sources is possible by modeling the distribution of particle size and shape parameters within a subsample. The analysis of more Al powder samples of different types and from a variety of manufacturers has generated a more robust data set for testing the scoring algorithms. Further, additional statistical approaches have been developed and tested on these high-dimensional, large data sets, which are not only suitable in the discrimination of Al powder sources, but which may be applicable to the interpretation of other high-dimensional forensic data, such as those encountered with trace evidence analyses.<sup>1,2</sup>

Using a variety of statistical approaches (various machine learning methods applied to summary statistics of subsamples and score-based methods for measuring the dissimilarity between distributions of particles), empirical tests have thus far shown that the applied methods have the ability to characterize the type of powder based upon the 17 measured size and shape parameters. Further, results have shown that, in most instances of incorrect classification of a subsample to a specific source, the misclassification is attributed to another source of the same general type (e.g., ball-milled foil subsample from source A is misattributed to ball-milled foil subsample from source C). The performance of the pairwise scores are evaluated based on the performance of the corresponding 1-nearest neighbor classifiers; various trials utilizing different portions of the collected data were implemented to determine which factors affected results and how those findings may allow for improvement of the analytical and interpretative methods. The use of automated microscopical techniques, in combination with image processing and various statistical methods, will allow for more efficient characterization and source discrimination of Al powders in IEDs, thereby providing valuable investigative lead and intelligence information.

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

- <sup>1.</sup> Kubota, N. 2002. *Propellants and Explosives: Thermochemical Aspects of Combustion*. Wiley-VCH Verlag GmbH, Weinheim.
- <sup>2.</sup> Conkling, J.A.; Mocella, C.J. 2011. *Chemistry of Pyrotechnics: Basic Principles and Theory*. CRC Press Boca Raton, Florida.

### **Aluminum Powder, Improvised Explosive Devices, Micromorphometric Analysis**

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## **B105 The Impact of Ultraviolet (UV) Radiation and Plane Polarized Light (PPL) Microspectrophotometry (MSP) in the Characterization and Differentiation of Deeply Colored Automotive Fibers**

*Andra Lewis, MSFS\*, Sam Houston State University, Huntsville, TX; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** After attending this presentation, attendees will gain knowledge and understanding about the strengths of light microscopy in combination with different capabilities of microspectrophotometry (MSP) including ultraviolet (UV) and plane polarized light (PPL) in the examination of deeply colored automotive carpet fibers which appear macroscopically similar in color.

**Impact Statement:** This research will impact the forensic science community by providing an understanding of the combined strengths of light microscopy and variants of MSP in the ability to differentiate deeply colored textile fibers. Studies have shown the inclusion of ultraviolet (UV) radiation as well as plane polarized light (PPL) provides greater discriminatory capabilities beyond the visible range.

The examination of textile fibers can be time consuming and tedious. However, they continue to prove their effectiveness in addressing questions about source attributions and the development of investigative leads in serious criminal cases. Fibers from carpeting and rugs found in homes and vehicles are commonly recovered as evidence because they are easily transferred between the scene(s) and both the victim and suspect. Although most carpet fibers found in homes are made of either nylon, polypropylene, or polyester, with differing cross-sectional shapes, automotive carpet fibers found in the trunk and passenger compartments of vehicles are made from a variety of substances. Due to demands to lower CO<sub>2</sub> emissions by reducing vehicle weight, automotive carpet fibers are being made from lighter substances known as nonwovens. Nonwovens in combination with other woven or knitted textile materials provide a significant contribution in this area. Many automotive carpet fibers are made from recycled polyester derived from plastic bottles as well as automotive carpet samples which are blends of several types, including polyamides (nylon 6, nylon 6,6, etc.), polypropylene (PP), polyester (PET), and polyolefins which can make the spectral analysis of these fibers challenging. A typical fiber examination starts with light microscopy for identification purposes followed by visible MSP and/or TLC. It has been shown incorporating ultraviolet radiation can provide further discriminatory factors and more recent studies have shown the discriminating potential of dichroism (plane polarized light or PPL) in conjunction with visible MSP. Furthermore, dichroic ratio measurements have the advantage of being independent of uncertainties due to optical path length (sample thickness) and pigment or dye concentration differences. Considering the current trends in the forensic science community toward method standardization and standards development, this research aims to determine the most efficient methodology for the application of microspectrophotometry (MSP) to the forensic differentiation of textile fibers applied to challenging fiber samples. The purpose is to identify objective criteria to develop a protocol for the capture, processing, and interpretation of spectral patterns for fiber specimens encountered in casework. In this research, forty (40) dark colored and macroscopically similar automotive carpet fibers were analyzed using microscopical examinations including color assessment, cross-sectional shape, and fluorescence followed by few alternative applications to visible MSP. The microspectrophotometer used in this research was not only equipped with full polarizing capabilities (i.e., polarizing filter and a rotating stage), but the analyzer and polarizer transmitted UV radiation down to 240nm, making pairing of the ultraviolet with plane polarized light a reality. The results of this research showed ~70% of the samples provided further discriminatory information through the combination of both ultraviolet radiation and plane polarized light to the characterization and differentiation of these fibers.

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### **Trace Evidence, Fiber, Ultraviolet-Visible Microspectrophotometry**

**B106 Using Capillary Columns for Measurements of Relative Distribution Constants of Vaporized Hydrocarbons Between Headspace and Polydimethylsiloxane (PDMS) Solid-Phase Microextraction (SPME) Fibers for Quantitative Chemical Analysis of Ignitable Liquids**

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**Learning Overview:** Attendees will expect to know a method of the estimation of true compositions of volatile compounds such as ignitable liquids in headspace via solid phase microextraction (HS-SPME) and capillary column gas chromatography (CCGC). The compositions of volatile compounds in headspace (HS) analyzed by a solid phase microextraction (SPME) sampling method usually differ from true compositions due to the difference in the distribution constants of these compounds between headspace and a polydimethylsiloxane (PDMS) SPME fiber are not equal. In this presentation, an analytical strategy to estimate relative distribution constants of vaporized hydrocarbons between air and PDMS using capillary column gas chromatography (CCGC) will be presented.

**Impact Statement:** This presentation will impact the forensic science community by proposing a way to estimate true compositions of vaporized hydrocarbons in headspace using relative distribution constants via capillary columns. The results produced in this work and future studies can be applied to the quantification of target volatile/semi-volatile compounds such as gasoline residues and other ignitable liquids present in headspace containing various types of forensic samples. This is important to identify the nature of ignitable liquids as well as their origins in forensic fire investigations.

For a quantitative chemical analysis of vaporized hydrocarbons in headspace via solid phase microextraction (SPME), distribution constants ( $K_D$ ) of these hydrocarbons between a SPME fiber and air are required. Recently, the relative distribution constants of three vaporized hydrocarbons between air and polydimethylsiloxane (PDMS) were determined by packed column gas chromatography (PCGC) in order to calculate headspace hydrocarbon compositions. However, preparation of packed columns is a cumbersome process in order to prevent possible experimental errors. Especially, a solid substrate should be completely and uniformly covered with a proper thickness of PDMS layer. In this regard, commercially available capillary columns with evenly coated polymeric stationary phases are proposed as alternatives to packed columns despite the limited selection of stationary phase.<sup>1-3</sup>

This study focused on the measurements and validity of relative distribution constants of vaporized hydrocarbons between air and polydimethylsiloxane (PDMS) using commercially available capillary columns. Capillary column gas chromatography (CCGC) measurements, using two columns containing a PDMS stationary phase with different film thicknesses (1 and 4  $\mu\text{m}$ ), were conducted to determine the relative distribution constants of *n*-heptane, toluene, *n*-octane, *p*-xylene, *n*-nonane, and 1,2,4-trimethylbenzene between air and PDMS at 90 and 120 °C. To validate the accuracy of the relative distribution constants via CCGC, the compositions of three headspace samples containing different amounts of hydrocarbons were calculated using the relative distribution constants via CCGC and extracted amounts via PDMS solid phase microextraction (SPME) at 90 and 120 °C.

To validate the relative distribution constants via CCGC, calculated headspace hydrocarbon compositions were compared to true headspace hydrocarbon compositions via the Wilcoxon signed-rank test (the significance level of 5%). A calculated *p*-value greater than 0.05 indicates there is no difference between the two compared data sets. All *p*-values calculated from the two data sets were greater than 0.05 and ranging from 0.5625 to 1. Therefore, calculated hydrocarbon compositions were close to experimental hydrocarbon compositions for all three samples at both 90 and 120 °C. In addition, calculated headspace hydrocarbon composition via two sets of relative distribution constants measured using two different capillary columns (1 and 4  $\mu\text{m}$  film thickness) were comparable as indicated by the *p*-values.

Finally, relative errors of calculated hydrocarbon compositions in absolute forms from two methods, CCGC and PCGC, were compared via the Mann-Whitney-Wilcoxon test with the bootstrap samples where the bootstrap method was considered to avoid any statistical parametric assumptions. The averages for absolute relative errors (%) in calculated hydrocarbon compositions via CCGC with 1  $\mu\text{m}$  PDMS film thickness, CCGC with 4  $\mu\text{m}$  PDMS film thickness, and PCGC were 3.1, 3.2, and 4.7, respectively. The bootstrap *p*-values are 0.914 and 0.902 from testing CCGC (1  $\mu\text{m}$ ) and PCGC and testing CCGC (4  $\mu\text{m}$ ) and PCGC, respectively. These results strongly indicate that relative errors of calculated headspace compositions measured via capillary columns are comparable to those via PCGC in the previous work. The results suggest that CCGC can provide a convenient and reliable method for measuring the relative distribution constants for the quantification of headspace containing various hydrocarbons via a HS-SPME sampling method using PDMS fibers.

**Reference(s):**

1. J. Pawliszyn. Quantitative aspects of SPME. In: J. Pawliszyn (Ed.), *Application of solid phase microextraction*, Cambridge, UK, RSC, 1997, pp. 3-21.
2. J. Kim; J. Cho; M. Schmitz; Z. Al-Saigh. Quantitative chemical analysis of volatile compounds via headspace-solid phase microextraction (HS-SPME) coupled with inverse gas chromatography (IGC). *Forensic Chem.* 11 (2018) 7-14.
3. J. Kim; J. Yu. Measurements of relative distribution constants of vaporized hydrocarbons between air and polydimethylsiloxane via capillary columns for the calculation of headspace hydrocarbon compositions. *J. Chromatogr. A* 1627 (2020) 461405.

**Distribution Constants, Solid Phase Microextraction, Quantitative Chemical Analysis**

## **B107 Evaluation of a Thermodynamic Model to Predict the Weathering of Ignitable Liquids at Different Temperatures**

*Hannah L. McMillen\*, West Virginia University, Morgantown, WV; Glen P. Jackson, PhD, West Virginia University, Morgantown, WV*

**Learning Overview:** After attending this presentation, attendees will understand how to apply fundamental principles of physical chemistry to mathematically model the weathering of ignitable liquids like gasoline.

**Impact Statement:** The thermodynamic model will help analysts to make more informed decisions about the temperatures and timeframes under which casework samples are likely to have been exposed. For example, the model can help tell the difference between liquids that have undergone slow evaporations at room temperature versus fast evaporations at elevated temperatures. The model can also be applied to predict a wide range of weathered chromatograms from a single chromatogram of an unweathered sample, which can facilitate the detection of ignitable liquid residues in casework samples without the need to collect an extensive GC-MS database of weathered samples.

Analyses involved the use of a local E85 gasoline that was weathered to a variety of different percentages. An aluminum block with a pre-weighed, clean aluminum weigh boat in the center was heated in an oven to the desired temperature, such as 30.0°C or 120.0°C. A volume of 1 mL of gasoline was transferred into the aluminum weigh boat, in the oven, and allowed to evaporate at a constant temperature for the desired duration(s). No air stream or vacuum was used to facilitate evaporation. A cold aluminum block was used to cool the aluminum weigh boat after being removed from the high temperature block. Weathering times ranged from 0.2 to 25 minutes.

The ignitable liquid residue (ILR) samples, diluted to a 1:200 dilution factor, were run on a Gas Chromatography-Mass Spectrometer (GC-MS). These results corroborated the current understanding of the weathering process at both low (30%) and high (95%) weathering percentages. The results were then compared to the thermodynamic model based on Raoult's and Dalton's laws.

Pearson Product Moment Correlations (PPMCs) determined the chromatographic similarity between the experimental data and the modeled data. For example, at 30.0°C, the best PPMC values between the modeled and measured chromatograms are on the order of 0.98 or better. However, the modeled chromatograms that provided the best (largest) PPMC values are not always the modeled chromatogram with the same percent weathering as the measured chromatogram: the average difference between the experimental and modeled extent of weathering using PPMC as the measure of fitness is 6.4% percent weathering (N=21 chromatograms predicted between 20-99% weathering). When using the PPMC values between experimental and modeled chromatograms, the error in predicting the extent of weathering of gasoline ranges from 0.37% to 11.2%, depending on the extent of weathering. The model works equally well at the elevated temperature of 30.0 °C.

The mean of absolute residuals between measured and modeled total ion chromatograms (TICs) were also calculated to determine the dissimilarity between the experimental data and the modeled data. The range of absolute differences between experimental and modeled weathering percentages is 1.06% to 18.06% at 30.0°C, and the mean error is 8.0%. In summary, the approach using PPMCs instead of mean absolute residuals provided more accurate predictions about the extent of weathering of the gasoline sample. Additional details in the presentation will include the accuracy of weathering predictions at elevated temperatures (120.0°C) and in the presence of porous substrates, which act to suppress the evaporation of volatile components in the gasoline and make the residues appear to be less weathered than would ordinarily be expected.

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### **Ignitable Liquids, Weathering, GC/MS**



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## **B108 Quantitative Ballistic Cartridge Case Analysis using Micro-CT**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the application of micro-CT to ballistic analysis of cartridge cases

**Impact Statement:** This presentation will impact the forensic science community by introducing a new methodology for forensic ballistic analysis

Forensic ballistic analysis requires the examination of fired ballistic components to try to establish information on the weapon that fired them. One feature on cartridge cases which is known to be representative of the weapon is the firing pin impression. This poster proposes micro-CT as an imaging technique for the 3-dimensional visualization of cartridge cases and quantification of firing pin impressions. The study consists of two parts: assessment of the applicability, reliability, and repeatability of micro-CT for cartridge case analysis; and comparison of cartridge cases fired from three weapons to establish the magnitude of variation within and between weapons.

A total of 72 cartridge cases fired by three distinct weapons were collected and micro-CT scanned to 5  $\mu\text{m}$  resolution. One randomly selected cartridge was scanned ten times under the same conditions to ensure repeatability of the scanning conditions. Following this, part two of the study introduced two cylindrical measurements are proposed to assess how repeatable the method is, and three functional firing pin impression measurements are proposed to establish the variability within and between weapons.

Micro-CT was found to be an effective and highly repeatable method for 3-dimensional imaging and measurement of ballistic cartridge cases. Furthermore, high agreement for inter-rater reliability was found between five raters. Quantitative micro-CT analysis of the functional firing pin impression measurements showed a significant difference between the studied weapons using Welch's t-test ( $p < 0.01$ ). Combination of the three measurements also showed a visible clustering separation between weapons suggesting a high applicability of the method should a large foundational database be pursued.

This study shows the advantage and reliability of utilizing micro-CT for firing pin impression analysis. Quantitative analysis of the firing pin impression allows distinction between the weapons studied. With expansion to further weapons, application of this methodology could complement current analysis techniques through classification models or statistical prediction.

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### **Ballistics, Micro-CT, Quantification**

## B109 An Internal Validation Study of the TopMatch 3D Scanner for Cartridge Cases

Kayli Carrillo, BS\*, Sam Houston State University, Huntsville, TX; Alexis Moser, BS, Harris County Institute of Forensic Sciences, Houston, TX; Jennifer Turner, MS, Harris County Institute of Forensic Sciences, Houston, TX

**Learning Overview:** After attending this presentation, attendees will be familiar with the TopMatch 3D Scanner's ability to obtain topographical details from cartridge cases.

**Impact Statement:** This presentation will impact the forensic science community with emphasis on firearms and toolmark examiners by demonstrating the application of virtual comparison microscopy through use of the TopMatch system in firearms identification laboratories.

The emergence of 3-dimensional (3D) surface topographies and computer algorithms for fired ammunition comparison has been termed Virtual Comparison Microscopy (VCM). VCM shows potential advantages over traditional microscopy when archiving and comparing evidence, training examiners and documenting comparisons and verifications.<sup>1</sup>

Currently, the TopMatch™ system is the fastest and highest resolution 3D scanner for VCM applications. It is also the first and only 3D scanner and analysis system to be validated for VCM by the FBI.<sup>2</sup> One of the goals of high-resolution 3D acquisition technologies is to overcome limitations associated with traditional comparison microscopy, especially those related to lighting conditions. To address challenges with lighting and reflection, TopMatch™ system utilizes GelSight™, a gel of optically clear elastomer with a thin layer of elastic paint on one side. The thin layer of paint conforms to the shape of the surface and removes unwanted surface reflectance properties to observe the head of the cartridge case. Several regions of interest such as the breech face and aperture shear are used by novel feature-based scoring algorithms to yield match scores between 0-1.<sup>2</sup>

Internal validation was conducted before using a TopMatch™ system in casework. This 3D surface topography technology has already gone through several validations and has demonstrated to meet or exceeded the performance of equipment used in traditional light microscopy.<sup>1-3</sup> The internal validation at Harris County Institute of Forensic Sciences included three phases. In Phase I, eleven previously analyzed proficiency tests were scanned and completed by analysts. In Phase II, three test fires shot from a Hi-Point Model C-9 were collected from a series of ten known consecutively manufactured slides were analyzed. In Phase III, eight lab generated test fires from the laboratory reference gun collection were evaluated. The breech face impression and aperture shear marks were measured at approximately 1.8 micron/pixel lateral resolution and sub-micron depth resolution. The ability for the analysis software to be remotely installed on each examiner's computer allowed for ease of use. With past proficiency tests, all examiners answered correctly.

### Reference(s):

1. Results of the 3D Virtual Comparison Microscopy Error Rate (VCMER) Study for Firearm Forensics. Chapnick, Chad, et al. s.l. : *Journal of Forensic Science*, 2020.
2. Introduction and Initial Evaluation of a Novel Three-Dimensional Imaging and Analysis System for Firearm Forensics. Weller, Todd, et al. 4, s.l.: *AFTE Journal*, 2015, Vol. 47.
3. Development and Validation of a Virtual Examination Tool for Firearm Forensics. Duez, Pierre, et al. s.l. : *Journal of Forensic Sciences*, 2017.

### Firearms Identification, Virtual Comparison Microscopy, Validation

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## **B110 The Assessment of Modern Glass from Portable Electronic Devices and Their Accessories by $\mu$ -XRF for Their Use in Forensic Investigations**

*Oriana Ovide, BS\*, West Virginia University, Morgantown, WV; Ruthmara Corzo, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV*

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**Learning Overview:** During this presentation, attendees will learn about the  $\mu$ -XRF elemental profile of modern glass from portable electronic devices (PED) and their glass accessories that can become valuable evidence in crime scenes.

**Impact Statement:** Given the high use of PEDs in modern lifestyles, this type of glass is anticipated to be found more often in crime-related incidents. Therefore, this study aims to expand the limited knowledge about their elemental composition and sources of variation within and between items. The forensic science community can use this study as a basis for further research into the potential value of glass from PEDs in casework.

Glass is a common type of trace evidence often found at crime scenes that can provide important information about the type and origin of the broken glass. Glass items that are commonly involved in crimes are typically soda lime glass, which has been studied extensively and for which there are current consensus standards available. Over the past decade, the prevalence of portable electronic devices (PED) such as phones, electronic watches, and tablets has increased. Since PEDs are designed to be kept close to a person, there is the potential for the transfer of broken glass from PEDs to become relevant evidence.

The recent demand for PEDs has led to constant upgrades to their components, including the glass screens of most PEDs. Currently alkali aluminosilicate glass is common and is defined by high alkali (typically Na) that will be replaced by a larger alkali element (typically K) to increase the strength and scratch resistance of the glass. To provide additional protection to their devices, individuals may also apply a tempered glass screen protector or, more recently, a liquid glass screen protector to their device.

In this study, both glass surfaces (inner and outer) from 30 different original PED screens, 15 different tempered glass screen protectors, and 5 PED screens to which 3 brands of liquid glass had been applied were analyzed using  $\mu$ -XRF following some modifications to the ASTM E2926 method (e.g., adapted to different elements of interest and comparison method due to the substantial difference to soda lime glass). A micro-XRF with two Silicon Drift Detectors (SDD) was used, taking 5 replicates per sample.

Examination of the 30 PEDs revealed high potassium, and the samples were classified into 5 groups based on their distinctive elemental profiles. Group one and two both had low zirconium and either had phosphorus or sulfur present in addition to aluminum, and iron. Group three and five had higher zirconium but group five also had titanium. Finally, group 4 had significantly higher Zn levels. Further examination of within-group samples through spectral overlay demonstrated good discrimination power (>99%), and for most samples, the top and bottom of the fragment could not be differentiated (76%). When the top and bottom of the fragment were deemed different, this was only by a slight difference in K, while the remaining relative elemental composition was indistinguishable. Additionally, spectral angle contrast (SAC) ratios were used as a more objective means to estimate the similarity between samples. Overall, SAC ratios between the top and bottom fragments of the same screen were low, ranging from 1 to 9. Conversely, ratios within groups were higher (ranging from 1 to 80) and ratios between different groups were much higher ranging from 10 to 460. The use of SAC ratios provides a proxy for the level of similarity between spectra rather than a binary decision.

The spectra from the 15 tempered glass screen protectors were differentiated into 4 groups based on their elemental composition. While group two represented samples most similar to soda lime glass, the remaining groups showed spectra similar to the PED glass. Additionally, spectra of just the liquid glass residues indicated that the major elements present within the sample were silicon, sodium, calcium, and potassium. However, the increase of the overall elemental profile for these elements in PED fragments with liquid glass was not significant.

This study provided support that PEDs and tempered glass screen protectors have the potential to be discriminated between different sources. This study provides a preliminary understanding of the elemental composition of these modern glasses and explores the discrimination capability of  $\mu$ -XRF for the forensic analysis of PEDs.

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### **PED Glass, Screen Protector Glass, $\mu$ -XRF**

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## B111 A Microscopical Examination and Elemental Analysis of Automotive Lubricating Greases

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**Learning Overview:** After attending this presentation, attendees will gain a greater appreciation for the expansion of potential evidential value to automotive greases.

**Impact Statement:** This presentation will impact the forensic science community with emphasis to the trace evidence community by expanding the consideration of new types of trace materials to automotive greases and show their potential contribution to forensic investigations.

Automotive greases may be recovered as stains on the ground at a road accident scene or on the garments of a suspect or victim allegedly involved in events under investigation. Numerous studies have been performed differentiating automotive motor oil for forensic applications, however little research was conducted to characterize or differentiate automotive greases. This study aims to determine the physical and chemical properties that allow a reliable identification and differentiation of automotive lubricating greases. It also aims to expand the consideration of potential trace materials that could help investigators or forensic laboratory personnel in the commission of crimes involving vehicles.

Thirty-five brand new commercially available grease samples were examined using light microscopy for potentially discriminating features. This initial microscopical examination showed the presence of microscopic particles that were part of the original formulation of the grease samples. These particles differed considerably in terms of shapes, sizes, and concentration between the different grease samples: Three particle types were identified and consequently classified as opaque, isotropic, and anisotropic.

Initially, the use of Fourier Transform Infrared (FTIR) spectroscopy yielded less than ideal data for these grease particles. Attempts were then made to characterize the observed particles using scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM/EDS), and this also did not yield informative results due to sample charging and oversaturation of the detector. Hence, an extraction procedure of these particles from the grease medium was necessary and an oil-solid separation method was developed based on the investigation of three solvents.

The solvents chosen were two petroleum solvents (hexane and pentane) and one green solvent (D-limonene). The ability of these solvents to dissolve the greases was studied by submerging chosen greases with an appropriate amount of each solvent for 15 minutes. These solutions were then gravity filtrated to separate out the particles, and air dried. Microscopy on the dried separation products revealed that the extraction achieved the removal of most of the base oil and left all three particle types intact. Based upon the results of the extraction, microscopy, and other green chemistry concerns, the solvent chosen for further extractions was D-limonene.

The visualization of these particles and the determination of their elemental composition was completed after extraction proved successful. Targets were selected for EDS analysis based upon SEM images using the backscatter detector and the particle size. It was discovered that the opaque particles analyzed are solid lubricant additives within the greases, containing various amounts of molybdenum and niobium. Greases within the sample set that do not contain opaque particles, are missing these heavy elements. These differences in composition display potentially discriminating properties for automotive greases.

This study demonstrates the efficiency of the extraction method developed and shows that automotive lubricating greases have high discriminating capabilities due to the variation of the elemental composition of the particles in the samples analyzed.

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**Forensic Science, Automotive Lubricating Greases, SEM/EDS**

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## **B112 Rapid Spectrochemical Mapping Techniques for Enhanced Detection and Visualization of Gunshot Residue Patterns**

*Courtney Vander Pyl, MS\*, West Virginia University, Morgantown, WV; Korina Menking-Hoggatt, PhD, West Virginia University, Morgantown, WV; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV*

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**Learning Overview:** The attendees will learn about the capabilities of LIBS and LA-ICPMS to offer rapid and reliable elemental information in the analysis of firearm-related evidence. Furthermore, they will learn the advantages of using laser-based methods for the visualization of GSR patterns of multiple crime scene substrates compared to the other methods currently in use.

**Impact Statement:** Utilizing rapid, laser-based methods for detection and interpretation of gunshot residues is anticipated to modernize current methods and aid fast decision making and more objective evidence assessment needed during the investigation and interpretation of firearm-related crimes.

Upon discharge of a firearm, microscopic particles consisting of inorganic species from the primer cap, known as inorganic gunshot residue (IGSR) are deposited on several surrounding surfaces. Likewise, some visible partially burn propellant flakes and other organic compounds (OGSR) are transferred. Receiving surfaces can include the shooter's hands and face, as well as target materials such as clothing, a wall, or a window. Conventional methods used in these investigations include Scanning Electron Microscopy-Energy Dispersive X-ray Spectrometry (SEM-EDS) for single particle analysis and colorimetric assays that react with organic and inorganic components of ammunition for distance determination. However, these techniques have some limitations. SEM-EDS is reliable, but it is time-consuming and costly. Colorimetric assays have severe drawbacks regarding limited selectivity and sensitivity, it is invasive, requires hazardous reagents and is highly subjective.

As a result, this study aimed to develop rapid chemical mapping methods for GSR pattern visualization using two laser-based methods: Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation-Inductively Couple Mass Spectrometry (LA-ICPMS). LIBS methods were developed and validated for shooting distance determination and bullet hole identification on complex substrates commonly found at firearm-related crimes. Residues were analyzed off 133 fabrics (white, dark colored, patterned, bloody), glass, drywall, and wooden samples. Spectrochemical data produced permanent 3D heat maps to visualize GSR patterns on surfaces. For shooting distance estimation, calibration curves were created for items shot at known distances (contact, 6 inches, 12 inches, 24 inches, and 36 inches). Additional test samples were shot at known distances but the ground truth was kept blind to the examiners. Principal Component Analysis (PCA) and Regularized Discriminant Analysis (RDA) were used to determine the distance. For comparison purposes, clothing samples were subjected to colorimetric testing (sodium rhodizonate and Griess). Color tests resulted in a misclassification rate of 26%, particularly on bloody and dark fabrics, while the LIBS method correctly classified the distance range of all unknown testing samples. For bullet hole identification, 21 difficult substrate materials were shot from a close distance, and the resulting GSR were transferred to an adhesive sheet, then mapped by LIBS to identify if a hole as produced by a bullet.

Additionally, a micro-spatial mapping method using combined LIBS and LA-ICPMS was developed for micro-bulk detection of IGSR collected from a suspected shooter's hands, providing simultaneous elemental profiles for particle(s) identification in the stub, at 25 discrete micro-regions. First, characterized IGSR suspensions (*p*GSR) created using 8 different primer types, 4 leaded and 4 non-leaded were used as "ground truth" compositions to test the methods. Sixty-four known-shooter samples (37 leaded and 27 non-leaded), and 60 non-shooter samples were then analyzed to test the mapping methods' abilities to correctly identify IGSR from shooter samples. Furthermore, correlation and covariance matrices were created using elemental intensities collected from *p*GSR, shooter, and background samples to assess the relationship of IGSR elements relative to the population of interest and as a means to describe the confidence the elemental profile originated from GSR as opposed to other sources. LA-ICPMS' enhanced sensitivity resulted in 94% classification accuracy, while LIBS resulted in 88% accuracy.

Overall, laser-based methods applied in this study demonstrated the feasibility of rapid techniques to be used for elemental mapping of GSR substrates often submitted as evidence in crime laboratories. Laser-based methods provided enhanced detection and visualization of GSR patterns on difficult substrates as compared to conventional colorimetric methods and allowed for the application of multivariate statistical interpretations. Continued development of rapid techniques for enhanced detection and visualization of gunshot residues could aid fast decision making and objective assessment of the evidence needed during the investigation of firearm-related crimes and interpretation of evidence in court.

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### **Gunshot Residue (GSR), Laser Ablation, Multivariate Statistics**

## **B113 Combining Novel Sampling Techniques and Electrochemical Detection in Gunshot Residue Analysis for Bullet Hole Identification and Distance Determination**

*Kourtney Dalzell, BS\*, West Virginia University, Morgantown, WV; Courtney Vander Pyl, MS, West Virginia University, Morgantown, WV; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV; Luis Arroyo, PhD, West Virginia University, Morgantown, WV*

**Learning Overview:** After attending this presentation, attendees will understand the gaps in knowledge regarding bullet hole identification and shooting distance determination, which the presented alternative sampling methods and electrochemical analysis aimed to address.

**Impact Statement:** This presentation will impact the forensic community by describing innovative sampling and electrochemical methods for bullet hole identification and distance determination for screening in shooting related crimes. The sampling technique combined with electrochemical detection of inorganic and organic gunshot residue provide 3D chemical and spatial information.

Bullet hole and distance determination are two examinations under the large umbrella of firearm discharge residue (FDR) analysis that aid in a shooting event's reconstruction. The distributions of FDR around target surfaces can help answering questions like how far away the shooter was standing from the target or if a given orifice was created by a bullet. The current approaches for bullet hole identification and distance determination rely on visual and physical examination followed by colorimetric methods. The modified Griess and sodium rhodizonate tests assess the presence of some organic and inorganic GSR (OGSR and IGSR) such as nitrites and lead, respectively. While these examinations are straight forward, there are some challenges with performing these tests due to their subjectivity, lack of specificity and sensitivity, and difficulty to apply to larger or complex substrates.

A series of sampling strategies were investigated using the universal collection method of carbon adhesive stub. Method development of sampling techniques focused on different approaches that permitted a 3D chemical mapping of residues for bullet hole identification and distance determination applications. Two sampling techniques were compared for bullet hole identification: (1) a single carbon stub was utilized to sample in 4 spots around the bullet wipe and (2) handpicking approximately 5 GSR flakes from the surface for microscopic examination and electrochemical analysis. For distance determination, we investigated four types of sampling methods: (1) a single stub lifted multiple times in a 8 cm line moving away from the entrance orifice, (2) four stubs, each sampling a 2 cm interval area from the bullet wipe to 8 cm in a single line direction, (3) four stubs, each sampling a 2 cm interval from bullet wipe to 8 cm in a circular direction, and (4) four stubs, each sampling four spots in diamond shape around the bullet wipe a 2cm intervals from the bullet wipe to 8 cm away. For comparison purposes, five replicates were collected per sampling technique (30 samples total) on 100% white cotton fabric (8 ½ by 11 inches) which were shot at a fixed distance of 6 inches with a Springfield XD9 9mm pistol and manufactured leaded ammunition. Electrochemical analysis was utilized as the main analytical technique for evaluation of target organic compounds and metals. The electrochemical method adapted from Ott et al. allows for the simultaneous detection of lead, antimony, copper, 2,4-dinitrotoluene, diphenylamine, nitroglycerin, and ethyl centralite.<sup>1</sup>

Electrochemical technique for detection reached detection limits of 0.012 µg/mL for IGSR and 0.147 µg/mL for OGSR. The bullet hole identification method resulted in positive identification of IGSR (lead, antimony, and copper) and OGSR (nitroglycerin, diphenylamine, and ethyl centralite) analytes in addition to visualizing the partially burnt flakes. Distance determination methods provided accurate identification of GSR analytes as well as spatial information of the distribution of residues around the entrance hole using heat maps. Sampling method results demonstrated that the line method cannot extrapolate spatial information, the circle method collected too much FDR on the stub oversaturating the electrochemistry signal, while the interval line provided the best visualization of the FDR distribution pattern. Electrochemical detection yielded combined signatures of at least one IGSR and OGSR compound in every sample, increasing the reliability for identifying the residues as originating from GSR. This work provides a good proof of concept that electrochemical detection using these sampling methods can provide forensic laboratories with a more sensitive, specific, and reliable method that can be used at the crime scene and laboratory. Future work will focus on method implementation on authentic samples, interference testing, and confirmatory analysis of samples by other analytical methods.

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- <sup>1</sup> Ott, C.E.; Dalzell, K.A.; Calderón-Arce, P.J.; Alvarado-Gómez, A L.; Trejos, T.; Arroyo, L.E. Evaluation of the Simultaneous Analysis of Organic and Inorganic Gunshot Residues Within a Large Population Data Set Using Electrochemical Sensors. *J. Forensic Sci.* 2020, 65 (6), 1935–1944. <https://doi.org/10.1111/1556-4029.14548>.

### **Electrochemistry, Distance Determination, Bullet Hole Identification**

## B114 A Characterization of Mounting Media for Hair and Fiber Microscopy

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**Learning Overview:** After attending this presentation, attendees will understand various mounting media used for hair and fiber forensic analyses and how these different media compare in terms of color, setting time, media autofluorescence, hair and fiber sample autofluorescence suppression or enhancement, and mounting media absorption in the ultraviolet range.

**Impact Statement:** This presentation will impact the forensic science community by providing forensic trace examiners an updated and diverse pool of commercially available media from which to consider alternatives.

Currently, Permount™ is being used by several forensic laboratories as a semi-permanent mounting medium for hair and fiber samples, mainly because of its ideal refractive index and of its performance as a matrix for long-term storage.<sup>1-4</sup> In some cases, visible region (VIS-MSP, about 380nm – 700nm) spectral data is not sufficient to distinguish fibers. Microspectrophotometry in the ultraviolet region (UV- MSP, about 240nm – 380nm) must be conducted. To analyze the full ultraviolet spectrum, fibers must be removed from the Permount™-glass setup, washed, and remounted in non-permanent glycerin on expensive quartz slides. Since this significantly increases the risk of sample damage and loss, hair and fiber examiners prefer to avoid remounting and observing UV signals below 380nm to the best of its ability.

Possible alternatives to the mounting medium Permount™ were evaluated and characterized for suitability in the use of sample preservation, imaging, and color discrimination. Through the use of examiner surveys, vendor inquiries, and previous research articles, a literature review was performed to identify commercially available mounting media since the publication of a previous evaluation study by Wiggins and Drummond (2007).<sup>5</sup> Evaluation of features such as toxicity, handling, setting time, ease of retrieval, media autofluorescence, fiber autofluorescence suppression, refractive index, media absorption in the ultraviolet (UV) range and sample preservation were conducted on final candidate mounting media. Additionally, color of mounting media themselves as well as color discrimination of embedded fibers were characterized for each candidate.

In all evaluative tests, multiple candidates performed as well as or outperformed control mounting media. Aqueous mounting media overall performed well in the UV spectral range, but glass was the major limiting factor. The evaluated mounting media exhibited different advantages and disadvantages. Before any firm recommendations can be made, further comprehensive validations must be done to evaluate the long-term conditions and stability of water-based mounting media after several months of storage at room temperature and under highly non-temperature control conditions.

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### Fiber Analysis, Light Microscopy, Mounting Medium

## **B115 A Diagnosis of Abusive Head Trauma (AHT): The Role of Immunohistochemistry in a Multidisciplinary Approach**

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**Learning Overview:** Because of the difficulty in making the diagnosis AHT, this case appears useful because it comprehends the current methods of investigation based on a necessary multidisciplinary approach. It provides an objective post-mortem confirmation of the hypothesis made by the clinicians while the patient was alive. The immunohistochemical analysis was a fundamental step of the diagnostic process.

**Impact Statement:** This case will have an impact on the forensic science community because it represents the complete diagnostic process that every pathologist will face when working on a similar image during their career. By attending this presentation, the audience will gain insight into performing the technical assessments necessary to make the diagnosis of abusive head trauma.

**Introduction:** The diagnosis of abusive head trauma (AHT) represents a challenge for the Pediatrician and Forensic Pathologist. Because AHT is not characterized by pathognomonic signs, the diagnostic process must necessarily be supported by a multidisciplinary approach. Retinal hemorrhages are one of the core manifestations in maltreated children and are found in about 85% of cases.<sup>1,2</sup> Several pathogenetic mechanisms of retinal hemorrhages have been described, of which vitreoretinal traction is the most credible.<sup>3</sup> Second, retinal venous obstruction represents another etiologic mechanism.<sup>4</sup> However, it has been associated with accidental head trauma and may not be related to abusive head trauma. Therefore, to make a differential diagnosis between accidental head trauma and AHT, some specific characteristics of retinal hemorrhages should be evaluated, such as the localization, subtype, and number, as well as the presence of retinoschisis and retinal folds. In AHT cases, different types of hemorrhages have been described. In less severe cases they occurred within the posterior retinal region and were single layered, while in more severe cases the hemorrhages were multi-layered and spread to the ora serrata. Thus, the severity of hemorrhage is directly related to the severity of trauma.<sup>5,6</sup> Although in AHT the hemorrhages are usually bilateral, monolateral hemorrhage does not exclude the diagnosis. If the ophthalmoscopic evaluation reveals many, severe, multilevel hemorrhages distributed over peripheral areas of the retina, the etiology of which cannot be verified, the diagnosis AHT should be made until proven otherwise.<sup>7</sup>

**Case Report:** A 3-month-old girl was admitted to the emergency department of the local hospital in a state of cardiopulmonary arrest; external examination revealed no pulse, apnea, pallor, mydriasis, and cutaneous hematomas. The child was then intubated and transferred to the Intensive Care Unit, where a CT scan revealed skull fractures and cerebral edema associated with diffuse cerebral hemorrhage. The diagnostic framework was completed by examination of the fundus of the eye, which showed multiple retinal hemorrhages. After 12 days, the child died, and an autopsy was ordered. The examinations confirmed the lesions found during the diagnostic tests and allowed histological analysis of tissues. Histology of the encephalon showed diffuse axonal damage (DAD) and encephalomalacia, and immunohistochemical analysis of the eye showed strong positivity for glycophorin A antibodies.

**Discussion:** In the presented case, the autopsy was performed to confirm the diagnosis of AHT made before the death. Together with the histological examination, all the signs typically described for AHT were found. In particular, retinal hemorrhage was easily detectable on microscopic examination of the eye. Moreover, the medical history provided by the parents was unclear and could not explain the severity of the clinical findings. The evidence of retinal hemorrhage, alongside multiple skeletal and brain injuries, allowed health care providers to make a quick and accurate diagnosis of physical child abuse.

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### **Abusive Head Trauma, Retinal Hemorrhages, Immunohistochemistry**



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## B116 Visualizing Shed Skin Cells in Fingerprint Residue using Dark Field Microscopy

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**Learning Overview:** In evidence samples that contain a limited amount of DNA such as latent fingerprints, proteins in the form of cells may be used to supplement individual identification using single amino acid polymorphisms. This study explores the practical aspects of such investigation by examining how much proteins are present in a typical latent fingerprint.

**Impact Statement:** Everyday tools such as dark field microscopy can be effective for the visualization of skin cells in latent fingerprints, which can be used to non-destructively quantify the amount of proteins. These proteins can then be used to supplement STR profiling through single amino acid polymorphisms that infer SNPs.

This proof of concept study shows that dark field microscopy provides sufficient contrast for cell visualization in fingerprints with high sebum content. Although the application is limited to smooth surfaces that do not scatter light, such as polyethylene terephthalate (PET), it was able to measure the number of cells deposited within a fingerprint residue and the reduction in cell transfer with repeated skin contact. On a PET surface, at roughly 5 N of contact force, a typical finger transfers several hundred cells onto the surface. Over subsequent finger contacts onto a clean PET surface, this number decreased exponentially until a steady state was reached, which is characterized by the transfer of  $(78 \pm 36)$  cells, or  $(0.46 \pm 0.21)$  cells/mm<sup>2</sup> when normalized for fingerprint area. High uncertainty in cell transfer was due to: the highly variable nature of a human finger (where the number of loose cells vary from person to person and from day to day depending on what they touch); and difficulties in controlling the contact force and finger movement such as twisting during deposition (where twisting of the finger can expose a new patch of skin to the substrate, increasing the number of cell transfer). Plasma etching was also explored as an effective way to validate dark field microscopy for cell counting. Although limited to inorganic substrates due to etching effects, exposing the fingerprint for less than 10 min can remove a majority of the sebum while keeping the cells intact for a before-and-after comparison using light microscopy.

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### Cell Visualization, Fingerprint, Microscopy

## B117 An Evaluation of the Stability and Durability of an Inkless Method for Fingerprint Recordings

Sihai Li, BS\*, University of New Haven, West Haven, CT; Josep De Alcaraz-Fossoul, PhD, University of New Haven, West Haven, CT

**Learning Overview:** After attending this presentation, attendees will learn about the proposed inkless method that utilizes commercial thermal paper and alcohol-based hand sanitizer gel to obtain reference (exemplar) fingerprints and how environmental variables over time, such as temperature, affect the visual quality (clarity) of the resultant fingerprints.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the stability and durability of the proposed inkless method as a potential safer and inexpensive alternative to the conventional ink method.

Fingerprint identifications have been used in criminal investigations for decades due to their reliability.<sup>1</sup> The legibility and uniqueness of friction ridge skin recordings are critical for subsequent comparisons to questioned fingermarks located at crime scenes. Also, for discarding other impressions deposited by victims and personnel who have had legitimate access to the scenes as well as for the identification of deceased persons.<sup>2</sup> When practicing the conventional inking method, ridge skin patterns will be coated with black ink and then transferred on standard tenprint cards, leaving inconvenient stains on the donor's skin. On the other hand, when utilizing inkless electronic equipment, such as Live Scan, the financial cost may be too high for certain agencies. As a result, a low-cost, cleaner, and safer inkless method to obtain reference (exemplar) fingerprints is recommended.

Previous research has proposed and proven the potential of an inkless method for obtaining exemplars with commercial thermal paper and alcohol-based hand sanitizer gel.<sup>3</sup> The visual quality (clarity) of the resultant fingerprints was comparable with those obtained from the conventional inking method as assessed with two different systems: an adapted quality score (QS) scheme and a set of metric data provided by the FBI's Universal Latent Workstation (ULW).<sup>3</sup> Results demonstrated that the ridge clarity produced by the new method was influenced by the biological sex of donors (female vs. male). Most importantly, it could be used as an alternative for obtaining exemplars.<sup>3</sup>

In order to further explore the viability of the proposed inkless method, it was necessary to examine additional factors that may affect the degradation of the thermal paper substrate. The purpose of this project was to determine whether environmental factors, i.e., temperature (°C) and relative humidity (%RH), in combination with biological sex could influence the quality of ridges over time. In a practical setting, it was important to evaluate whether the expected degradation of thermal paper could influence the quality of fingerprint ridges and be mitigated by preserving it under colder temperatures. A total of 120 fingerprints were obtained from 9 females and 11 males. Inkless impressions of the index, middle, and ring fingers were deposited in duplicate, and each set was stored under different environmental conditions. One set was stored at an average room temperature of 21.4°C with a %RH of 42.5%; while the other was exposed to an average temperature of 2.9°C with a %RH of 12.2% for the first 22 weeks (fridge) and later moved to room temperature and %RH as with the other set. After aging over 72 weeks, impressions were photographed, images were standardized using image processing software and then analyzed with ULW. The clarity metric provided by ULW was used as reference to evaluate any visual differences between the two aging sets.<sup>4</sup> All samples were examined and compared in relation to time, temperature/relative humidity, and biological sex.

This research provided empirical evidence that the storage at colder temperatures and the time interval between depositions did not affect the quality of ridges on thermal paper significantly. Statistical tests proved the stability and durability of the proposed inkless method for fingerprinting purposes regardless of temperature and time. A biological sex effect was again identified where males showed better ridge clarity. This was consistent with the literature in which males and females have shown differences in the morphology of friction skin ridges that could affect the quality of ridges. In future work, longer storage periods, different temperatures and relative humidity may provide more insightful information on the validity of the proposed inkless method.<sup>5</sup>

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### Fingerprinting, Thermal Paper, Environmental Conditions

## B118 Standards Development Activities in Trace Materials

*Celeste M. Grover, MSFS\*, Oregon State Police, Forensic Science Division, Clackamas, FL*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards pertinent to the examination and interpretation of trace materials.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities related to trace material examination and interpretation. It will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards development organizations (SDOs) that ballot and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development activities in trace materials will be discussed. These include:

Recent standards that have been added to the OSAC Registry

- *E3260 Standard Guide for Forensic Tape Analysis and Comparison*
- *E3272 Standard Guide for Collection of Soils and Other Geological Evidence for Criminal Forensic Applications*
- *E2808 Standard Guide for Microspectrophotometry in Forensic Paint Analysis*

Published ASTM standards that have yet to go through the Registry approval process

- *E2224/WK58028 Standard Guide for Forensic Analysis of Fibers by IR*
- *E2225 Standard Guide for Forensic Examination of Fabrics and Cordage*
- *E2227 Standard Guide for Forensic Examination of Non-reactive Dyes in Textile Fibers by Thin Layer Chromatography*
- *E2228 Standard Guide for Microscopic Examination of Textile Fibers*
- *WK70035 Standard Practice for Determination and Comparison of Color by Visual Observation in Forensic Soil Examination*
- *WK72837 Standard Guide for Analysis of Forensic Geological Materials by Powder X-Ray Diffraction*
- *WK72932 Standard Guide for Forensic Glass Analysis and Comparison*
- *WK56743 Standard Practice for Training in the Forensic Examination of Hair by Microscopy*
- *WK72597 Standard Guide for the Forensic Examination of Hair by Microscopy*
- *E2809/WK57479 Standard Guide for Using Scanning Electron Microscopy/X-ray Spectrometry in Forensic Polymer Examinations*
- *WK74138 Standard Guide for Using X-ray Fluorescence in Forensic Polymer Examinations*
- *WK75180 Standard Guide for Using PyGC-MS in Forensic Polymer Examinations*

Documents currently in development at the OSAC

- *Standard Guide for Interpretation and Reporting in Forensic Comparisons of Trace Materials*
- *Standard Guide for Forensic Physical Fit Examinations*
- *Standard Practice for a Forensic Fiber Analysis Training Program*
- *Standard Guide for Forensic Fiber Analysis and Comparison*
- *Standard Guide for Forensic Analysis of Fibers by Microspectrophotometry*
- *Standard Practice for a Forensic Glass Analysis Training Program*

Priorities for new documents or work products

- *Standard Guide for Physical Fit Training Program*
- *Standard Guide for Using X-ray Diffraction in Forensic Polymer Examinations*
- *Standard Guide to Raman Spectroscopy in Forensic Polymer Examinations*
- *Standard Guide for Accessing Physical Characteristics in Forensic Tape Examinations*
- *Standard Guide for Using Light Microscopy in Forensic Tape Examinations*
- *Standard Guide for Using Scanning Electron Microscopy for Morphology and Energy Dispersive Spectroscopy in Forensic Soil and Geological Materials Analysis*
- *Standard Guide for the Use of Polarized Light Microscopy in the Forensic Examination and Comparison of Sands and Soils*

and other highlights. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

### Trace Materials, Standards, OSAC

**B119 Five Years Since the President’s Council of Advisors on Science and Technology (PCAST) Report: The Value of Materials Science and Tribology as Foundational Science for Transfer and Persistence**

*Michael Aberle, BA\*, National Centre for Forensic Studies, University of Canberra, Bruce, ACT, Australia; Hilton Kobus, PhD, National Centre for Forensic Studies, University of Canberra, Bruce, ACT, Australia and Flinders University, Bedford Park, SA, Australia; James Robertson, PhD, National Centre for Forensic Studies, University of Canberra, Bruce, ACT, Australia; Caroline O’Driscoll, PhD, National Centre for Forensic Studies, University of Canberra, Bruce, ACT, Australia and Australian Federal Police, Canberra, ACT, Australia; Jurian Hoogewerff, PhD, National Centre for Forensic Studies, University of Canberra, Bruce, ACT, Australia and Australian Federal Police, Canberra, ACT, Australia*

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**WITHDRAWN**

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## **B120 Forensic Discrimination of Copper Metal by Laser Induced Breakdown Spectroscopy (LIBS)**

*Chase Notari, BS\*, University of New Haven, West Haven, CT; Brooke Kammrath, PhD, University of New Haven, West Haven, CT and Henry C. Lee Institute of Forensic Science, West Haven, CT*

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of the capabilities and limitations of LIBS for the comparative analysis of copper metal, specifically the copper jacketing of bullets, and its capabilities as an investigative tool for use in forensic casework.

**Impact Statement:** This presentation will impact the forensic science community by presenting a novel application of LIBS for the discrimination of copper metal, specifically the copper-jacketing of bullets.

Copper metal has great potential as forensic evidence due to its presence in a range of cases from thefts of copper wiring and pipes, the use of copper wiring in IEDs, and its common function as jacketing for bullets. Copper has already been proven to be able to be discriminated through trace element profiles collected from ICP-MS, although there are instruments with faster analysis and no sample preparation that are being investigated use in forensic analysis, not only for copper, but for glass, paint, and other types of evidence as well.

LIBS is an analytical technique that has continued to gain prominence in the scientific community as a valuable tool for elemental profiling. In addition, it is advantageous when compared to other similar instruments due to the fact that it is rapid, requires no sample preparation, is able to simultaneously provide information on multiple elements at once, and is less expensive than other instruments used for elemental analysis. Furthermore, LIBS has already been tested to have value in the analysis of other types of forensic evidence such as paint, soil, and ink.

The goal in this research is to evaluate LIBS for its ability to comparatively analyze copper, focusing primarily on the copper-jacketing of different bullets. The study first explored the detection capabilities of the instrument, while also determining an appropriate element menu, and outlining the optimal parameters for the LIBS. Parameters were tested and optimized using a copper density block; parameters included laser pulse energy, spot size, pattern size, gate delay, number of pulses per shot, and repetition rate. Once determined, the optimal parameters were applied to the analysis of the copper jacketing from the different bullets, and results were analyzed using multivariate statistical methods (Principle Component Analysis, Canonical Variance Analysis, and Partial Least Squares Discriminant Analysis).

This research showed that there is great potential for LIBS to provide a novel method for the forensic analysis of copper metal, and specifically copper-jacketed bullets. The results demonstrate that elemental analysis by LIBS has the ability to differentiate bullets based on the elemental profile of their copper jacketing. Discrimination of bullets is improved when combined with LIBS elemental analysis of bullet lead. This research also provides evidence that other sources of copper may be able to be analyzed and discriminated using LIBS, thus extending this powerful tool to additional items of forensic relevance, such as pipes and wiring.

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### **Copper Metal, Bullets, LIBS**

## **B121 The Transfer of Metal Traces by Human Touch Detected Using a Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) Technique: A Pilot Study**

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**Learning Overview:** After attending this presentation, attendees will understand more about the transfer of metal traces by touch.

**Impact Statement:** This presentation will impact the forensic science community providing experimental data that can be extremely useful in a forensic setting, for instance when it is necessary to establish, for example, if a victim has been touched by someone who held a weapon or another metal object found on the scene.

The transfer of metal particles could be extremely useful in a forensic setting, for example metal residues can be transferred from metal weapons on the victim's skin. The presence of metal traces can be assessed using SEM-EDS (scanning electron microscope equipped with energy dispersive spectroscopy) analysis.<sup>1</sup> The studies in literature about metal traces detected using SEM-EDS focus especially on gunshot residues, metal particles in the "current mark", and traces on bone tissue after blunt and sharp injuries.<sup>2-6</sup> The transfer of metal particles between different surfaces hasn't been investigated until now. Aims: this pilot study aims to assess the entity of the transfer of metal residues by human fingertips from metal objects to different surfaces. Materials and methods: the experiment have been carried out as follows: (1) FIRST TOUCH SAMPLES: subject 1 (a volunteer) touched ten different metal items (8 things of everyday usage, 1 gun, 1 cartridge case) using both the thumb fingertips, applying pressure but no rubbing. Then, for each item a metal stub covered by a carbon tape has been applied on the fingertips and then analyzed using SEM-EDS. (2) SECOND TOUCH SAMPLES: after touching the ten items as explained above, subject 1 touches three different surfaces: glass, plastic and the skin of a second volunteer (subject 2). One sample has been taken from each touched surface, obtaining in total 30 "second touch" samples. The 40 samples have been observed using EM30X (COXEM), as well as fragments of the metal items used for the experiment. The observation was carried out using backscattered electrons, in order to detect only the inorganic particles; metal fragments appear bright using this setup, due to the higher atomic number. Results: the metal residues were found in the totality of the examined samples. The semiquantitative analysis shows a lower amount of traces in the "second touch" samples. The EDS analysis showed a high correspondence between touched items and primary metal fragments. Conclusions: the results above summarized allowed to demonstrate that, after touching a series of different metallic items, traces of them persist on the fingertip's skin and can be detected using SEM-EDS. Moreover, such traces can be transferred on a second surface and detected using the same technique. This presentation will impact the forensic science community providing experimental data that can be extremely useful in a forensic setting, for instance when it is necessary to establish, for example, if a victim has been touched by someone who held a weapon or another metal object found on the scene.

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### **Transfer by Touch, SEM/EDS Analysis, Metal Particles**

## **B122 A Comparative Study on the Background Presence of Glass and Paint in Various Populations and Seasons in the United States**

Lauryn Alexander, PhD\*, West Virginia University, Morgantown, WV; Oriana Ovide, MS, West Virginia University, Morgantown, WV; Olivia Duffett, BS, West Virginia University, Morgantown, WV; Andra Lewis-Krick, PhD, Sam Houston State University, Huntsville, TX; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV

**Learning Overview:** After attending this presentation, attendees will gain knowledge about the background presence of glass and paint in two U.S. cities and different seasons, according to the type of clothing and footwear worn, along with their retention characteristics.

**Impact Statement:** This presentation aims to fill the current gap of knowledge concerning the random presence of glass and paint within the United States' general population, and how different demographics and geographical aspects can impact the overall background levels of traces.

Glass and paint particulates are often recovered from crime scenes as a primary form of trace evidence that can provide information about how the events took place. Baseline background presence of these traces in the general population are essential in assessing their evidential value. Although studies have been performed on the background presence of glass and paint in areas such as Canada, Australia, the United Kingdom, and Northern Ireland, surveys are limited within the United States.<sup>1-9</sup> The available studies have drastically different demographics, socioeconomic statuses, and/or geographical circumstances, as well as methodologies, sample types, and experimental designs, making the use of background data challenging.

As a result, this collaborative study between West Virginia University and Sam Houston State University focuses on collecting glass and paint residues from volunteers with different demographics and socioeconomic backgrounds in various U.S. cities during two different seasons. The study provides valuable information on the chance of randomly finding glass and paint particles in the general population, as well as the recovery location from the type of clothing and footwear worn.

This presentation discusses the findings from samples collected in Morgantown, WV during the winter and summer seasons, as well from samples collected in Huntsville, TX during the spring season. One hundred volunteers with up to 6 separate garment areas per volunteer were sampled utilizing taping and scraping methods. Each sample was systematically searched, and any paint or glass particulates were identified. Glass fragments were analyzed via SEM-EDS and/or  $\mu$ XRF, as well as refractive index, while paint particulates were analyzed via micro-FTIR and micro-ATR techniques. The Morgantown set collected in the winter consisted of 511 items, in which 10 glass fragments (~2% of the sampled items) and 118 paint fragments (~20% of the items) were identified. In total, approximately 50% of the individuals sampled had paint fragments recovered from their clothing, while only 7% of individuals had glass on them. The Huntsville set collected in the spring consisted of 398 items, with 8 glass fragments (~2% of the items) and 93 paint fragments (~20% of the items) identified. In total, approximately 46% of the individuals sampled had paint fragments recovered from their clothing, while only 8% of individuals had glass on them. No individuals had both particle types on their garments. In both subpopulations, the majority of the paint fragments were recovered from the upper garments while most of the glass was recovered from the footwear sole. Overall, both Morgantown and Huntsville sets yielded close proportions of background presence of glass and paint particles.

The baseline data presented in this study will increase the background knowledge on the background presence of glass and paint in the United States and aims offering valuable information for the assessment of the evidential value of trace evidence in the US court system.

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### **Glass and Paint, Baseline Background, Collaborative Study**

**B123 An Analysis of Simulated Automotive Paint Smears Representing Vehicle-to-Vehicle Collisions Using an Infrared (IR) Imaging Microscope**

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**WITHDRAWN**



## B124 Interlaboratory Studies to Evaluate the Forensic Analysis and Interpretation of Glass Evidence

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of utilizing likelihood ratios (LRs) for the interpretation of forensic glass evidence.

**Impact Statement:** This presentation will impact the forensic science community by presenting an accessible way to calculate LRs, an alternative method for the interpretation of multivariate LA-ICP-MS data comparisons.

The results of two interlaboratory exercises involving 20+ operational and research laboratories employing laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and using a standard test method (ASTM E2927-16e1) for the forensic analysis and comparison of glass evidence are presented.<sup>1</sup> The overall aims of the first exercise were to evaluate the performance of a newly reported calibration standard (CFG2) for the quantitative analysis of simulated casework samples and to evaluate the use of a qualitative determination of the strength of association or non-association (as recommended by the ASTM E2927) and the use of a likelihood ratio (LR) as an objective and quantitative determination of the strength of any association found.<sup>2-5</sup> Ten (10) laboratories determined an LR for the comparisons to report the significance of glass source comparisons for a set of glass samples of known origin. Both the ASTM E2927 match criterion and previously reported LR calculations were used to interpret the results of the comparisons reported by the 10 different laboratories. Two different background databases were used for the calculation of the LR to gauge the effect of the size and nature of the databases on the calculation of the LR.<sup>6,7</sup> As expected, glass that originated from the same windowpane was found to be indistinguishable using the ASTM E2927 match criteria and result in a high LR value (suggesting strong support for an association between the glasses) and glass that originated from different vehicles are distinguished (resulting in a low LR, suggesting strong support for an exclusion).<sup>6,8,9</sup> Glass samples that originated from different vehicles but that were the same make, model, and year (comparisons between the inner and outer pane of the same windshield) were found to exhibit chemical similarity that is sometimes reflected in a low LR. Good agreement among the laboratories was also reported for the analytical data collected using the standard test method of analysis for reference materials and the samples analyzed with relative standard deviations < 5 % RSDs among all labs participating in the study with few exceptions.<sup>2</sup> The overall aims of the second exercise were to allow for all participants to gain proficiency in the free-available Shiny App, created by our group, to facilitate the calculation of likelihood ratios (LR), compare the likelihood ratios from different scenarios using a wide range of background databases from across the world, and interpret the data to provide recommendations to create a standardized interpretation method for the use of likelihood ratios for glass analysis, which will be submitted to ASTM.<sup>10</sup> The 20+ laboratories determined an LR for the three (3) different scenarios. Both the ASTM E2927 match criterion and previously reported LR calculations were used to interpret the results of the comparisons reported. Five (5) individual databases were used to gauge the effect of different sized non-local databases on LR calculations. Glass that originated from the same pane was found to be indistinguishable using the ASTM E2927 match criteria and result in a high LR value and glass that originated from different windows are distinguished.<sup>6,8,9</sup> Glass samples that originated from chemically similar windows were found to be reflected in LRs between the maximum and minimum.<sup>6,8,9</sup>

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### LA-ICP/MS, Forensic Analysis, Comparison

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## **B125 A Chemical Approach to Tire Mark Analysis—Preliminary Protocols From Collection on Scene to Elemental Profiling**

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**Learning Overview:** After attending this presentation, attendees will have an understanding of using lifting procedures, Raman microscopy, and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) to collect, extract, and analyze tire marks from road crime scenes.

**Impact Statement:** This presentation will impact the forensic science community by providing preliminary protocols to collect tire marks on the road, separate tire trace material from skid mark lifts, and establish their elemental profile with the potential to be analyzed for tire and skid mark association.

Road crime is an ever-present aspect of the forensic community, with a lack of quantitative tools in the investigation. One such area that is lacking is in tire mark investigations, where traces of tire evidence are present. Previously tire patterns have been used for linking tire and skid mark and thus car, but anti-lock braking systems making readily visible skid marks rare. However, tire traces are still left where the tire mark was made, meaning that they can be chemically or elementally analyzed to get information about the tire which left them.

In the United States between 2004 and 2018, 5% of fatal vehicle incidents were fatal hit and runs, where knowledge of the tire of the car is sometimes the only evidence available.<sup>1</sup> Even in cases where vehicles are still present, attribution of a tire mark can assist in accident reconstruction.

One challenge present with this route of analysis is that tire trace material must be collected from the road. Lifting the material from the road has many options, including gel lifting tapes, different casting compounds, or collecting trace material with tweezers directly from the road. These methods were tested and compared to give the best results for later analysis. Field tire marks evidence were created and lifted in collaboration with the Florida Highway Patrol.

Current methods of analysis for attribution involve either molecular or elemental signatures using a few different analytical techniques. Of particular interest to us, laser induced breakdown spectroscopy has been shown to be able to attribute bulk tires.<sup>2</sup> Both these methods need a reliable way to separate tire trace material from the lift while also differentiating what is just road material.

Once the lift is performed, separating those tire materials from other contaminants, mainly the asphalt, is a challenge that needs to be addressed. Some chemical separation methods have been tested, but ultimately present possible contamination of their own. In this study, Raman microscopy was used to differentiate the tire trace material from the asphalt. This was shown to easily differentiate asphalt from tire materials, allowing manual separation of the tire material from the tire mark lift.

After the tire trace is separated from the lift, the material was analyzed for elemental profiling using ICP-MS. By comparing the elemental signature of the tire trace and the bulk tire, the effect of transferring bulk tire to tire mark was examined. This step is important in knowing how the bulk tire will correlate with the tire trace when attribution of a skid mark to tire is desired.

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### **Tires, Trace Evidence, Raman Microscopy**

## **B126 Evaluating the Discriminating Power of Hair Amino Acid Ratios for Distinguishing Individuals Using Gas Chromatography/Mass Spectrometry (GC/MS)**

*Timothy Yaroshuk, MS\*, University of New Haven, West Haven, CT; Alyssa Marsico, PhD, University of New Haven, West Haven, CT*

**Learning Overview:** To understand the variability of amino acid ratios between different hair samples in order to differentiate individuals.

**Impact Statement:** This presentation will demonstrate the capabilities and limitations of using amino acid ratios in hair to differentiate individuals in cases where DNA analysis cannot be conducted.

Currently, the conventional methods of analyzing hair include microscopic hair comparison (MHC) and DNA analysis (nuclear and mitochondrial), with nuclear DNA analysis being individualizing. However, MHC is subjective and nuclear DNA analysis is not always possible if not enough adequate cells are present.<sup>1-3</sup> Non-synonymous amino acid changes in the hair protein sequence – resulting from single nucleotide polymorphism profiles that differ between individuals – can be exploited to offer supplemental or alternative routes for hair analysis. Currently, proteomics has successfully exploited genetically variant peptide (GVP) content in hair to differentiate at least non-related individuals.<sup>4-7</sup> However, proteomics is complicated and requires the GVPs to remain intact. Analyzing amino acids is an alternative method that may simplify the analysis. It has been demonstrated that analyzing amino acid quantities has abilities to differentiate people based on general class characteristics (sex, age group, and geographical origin).<sup>8</sup> A study by Macri et al., analyzing amino acid ratios of two individuals with morphologically similar hair, discovered 15 amino acid ratios that differed.<sup>9</sup> Expanding on this study, this research will evaluate the discriminating power of using hair amino acid ratios to differentiate individuals with a focus on increasing the sample size and diversity to develop a method that can supplement MHC in cases when DNA analysis cannot be conducted.

Hair samples were obtained from consenting individuals and were anonymized. Brushed hair, plucked hair or cut hair samples were obtained from 10 individuals, including a sample from one individual before and after dyeing their hair. Hairs were thoroughly washed with deionized water and methanol to remove surface contaminants. They were then cut into smaller pieces, hydrolyzed with hydrochloric acid for protein digestion in triplicate and subsequently filtered to remove unhydrolyzed hair pieces. An aliquot was then taken to dry under a gentle stream of nitrogen and L-norvaline was added as an internal standard. The sample was then reconstituted in ethyl acetate, and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added for amino acid derivatization. The samples, in addition to a set of 11 standard derivatized amino acids in ethyl acetate, were analyzed using gas-chromatography mass-spectrometry (GCMS).

Eight derivatized amino acids were detected from the hair samples in addition to a glutamic acid derivative. Identification confirmation was conducted by comparing sample retention times to standards as well as mass spectra library comparison. Quantitation relative to the internal standard was completed and 36 amino acid ratios were constructed from these quantities. Outliers for each ratio were determined by the Grubbs test and these were discarded. Based on preliminary comparisons of means and standard error of the ratios between individuals, individuals can be differentiated using hair amino acid ratios. Between the 10 hair samples analyzed, one-way ANOVA for all 36 ratios had  $p < 0.05$  indicating the ratio is different between at least 2 individuals. Most individuals were differentiable using the post-hoc Tukey test for all ratios. For the samples that could not be differentiated with this method, 3D-PCA plot showed distinct clustering of individuals, therefore allowing differentiation. Ratios between dyed hair samples were also differentiable from undyed hair from the same donor.

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### **Amino Acid, Gas Chromatography/Mass Spectrometry, Hair**

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## B127 Standards Development Activities in Seized Drugs

*Agnes D. Winokur, MS\*, Drug Enforcement Agency, Southeast Laboratory, Miami, FL*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline specific standards pertinent to examination of seized drugs.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to the examination of seized drugs. It will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in seized drugs will be presented. These include i) recent standards that have been added to the OSAC Registry (E1968-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Cocaine, E1969-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Methamphetamine and Amphetamine, E2125-19 Standard Practice for Microcrystal Testing in Forensic Analysis for Phencyclidine and Its Analogues, E2882-19 Standard Guide for Analysis of Clandestine Drug Laboratory Evidence, E2548-16 Standard Guide for Sampling Seized Drugs); ii) OSAC proposed standards (Standard Guide for Assessment of Fourier Transform Infrared (FTIR) Spectroscopy Data During the Qualitative Analysis of Seized Drugs, Standard Guide for Assessment of Gas Chromatography and Electron Ionization Mass Spectrometry Data During the Qualitative Analysis of Seized Drugs, Standard Guide for The Development of Electron Ionization-Mass Spectral Libraries for the Analysis of Seized Drugs, Standard Guide for Intra-laboratory Blind Quality Control Programs for Seized Drug Analysis); iii) documents currently in development at the OSAC or by the SDO (Standard Practice for the Differentiation of Marijuana and Hemp in Seized Drug Analysis, Standard Guide for Court Testimony in Seized-Drug Analysis, Revision of E2549 Practice for Validation of Seized Drug Analytical Methods into an Interdisciplinary Standard, Revision of E2764-11 Standard Practice for Uncertainty Assessment in the Context of Seized-Drug Analysis); iv) priorities for new documents or work products (Standard Guide on Reporting in Seized-Drug Analysis, Standard Practice for the Analysis of Opioids and Emerging Drugs, Standard Guide for the Assessment of Liquid Chromatography (LC) Data During Qualitative Analysis of Seized Drugs); and other highlights. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### Seized Drugs, Standards, OSAC

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## B128 A Comparison of Analytical Workflows for Seized Drug Analysis

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the qualitative and quantitative differences between two analytical workflows for seized drug analysis. One workflow incorporated color tests and generic GC-FID and GC-MS methods and the second incorporated DART®-MS and targeted GC-MS methods.

**Impact Statement:** This presentation will impact the forensic science community by providing both qualitative and quantitative measures of different screening and confirmatory approaches. This information can be used to make data-driven decisions on changes to workflows or adoption of new technology.

As the challenges presented by the continued presence of synthetic opioids, novel psychoactive substances, and other emerging drugs persist, laboratories are continuing to look for new analytical approaches or techniques to ease the burdens. These solutions range from modifications to existing methods or techniques to implementation of entire new technologies. Laboratories often must estimate the benefits of implementing changes making adoption difficult because there may be an incomplete understanding of how, and even if, these changes will address the challenges a laboratory is facing. One way to mitigate this issue is to develop metrics that compare different analytical approaches to provide laboratories and personnel with tangible data to make decisions.

In this study we attempt to compare, qualitatively and quantitatively, two analytical workflows for seized drug analysis using adjudicated and mock case samples containing synthetic cannabinoids, cathinones, and opioids. The first workflow, an existing workflow, employed color tests for screening followed by general-purpose gas chromatography flame ionization detection (GC-FID) and general-purpose gas chromatography mass spectrometry (GC-MS) analyses for confirmation. The second workflow was an experimental workflow that combined direct analysis in real time mass spectrometry (DART®-MS) for screening with targeted GC-MS methods for confirmation. Four forensic chemists were asked to analyze a subset of samples on each workflow and at each step in the analysis scheme, recorded metrics such as the time required, and level of information obtained.

Using the information from the study, the two workflows could be compared. A scoring system was developed to allow for comparison of the two screening approaches – DART®-MS and color tests. DART®-MS required the same amount of time as color tests but yielded significantly more accurate information. Confirmation of samples using GC-FID and GC-MS general purpose methods required more than twice the amount of instrument time and data interpretation time compared to targeted GC-MS methods. There were also several analytical challenges that prevented compound confirmation in some samples using the existing workflow. Targeted GC-MS methods were found to simplify data interpretation, reduce consumption of reference materials, and address almost all limitations of general-purpose methods. The results of this study highlight how rethinking processes for seized drug analysis could assist laboratories in reducing turnaround times, backlogs, and standards consumption.

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### Seized Drugs, GC/MS, DART®-MS

## B129 Evaluating the Robustness and Ruggedness of a Statistical Method to Compare Mass Spectra

*Andrew Sacha, BS\*, Michigan State University, East Lansing, MI; Victoria McGuffin, PhD, Michigan State University, East Lansing, MI; Ruth Waddell Smith, PhD, Michigan State University, East Lansing, MI*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the robustness and ruggedness of a method developed to statistically compare mass spectra, with a focus on differentiating structurally similar novel psychoactive substances.

**Impact Statement:** This presentation will impact the forensic science community by further demonstrating a more objective method that can be used toward seized drug identifications.

Identification of seized drugs in submitted samples often involves a visual comparison of mass spectra as one part of the analytical scheme. However, with the increasing prevalence of novel psychoactive substances (NPS), identification based on visual comparison of spectra can be challenging due to the high degree of structural similarity among compounds within given NPS classes.

Previous work in our laboratory developed a statistical method to compare two mass spectra and, thereby, increase objectivity in the comparison. The method is based on the unequal variance *t*-test, which is performed to compare the mean intensities of corresponding *m/z* values in the two spectra at a user-specified confidence level. The null hypothesis states that the difference in mean intensities at that *m/z* value is equal to zero, whereas the alternative hypothesis states that the difference is not equal to zero. If the null hypothesis is accepted at all *m/z* values in the spectral range, the two spectra are considered to be statistically equivalent. In contrast, if the null hypothesis is not accepted at any *m/z* value, the two spectra are considered to be statistically distinct. In these cases, the *m/z* values for which the null hypothesis is not accepted are termed 'discriminating ions' and the number of such ions is determined. The method has been applied previously to demonstrate successful association and discrimination of structurally similar seized drugs and positional isomers.

The next step is to demonstrate the robustness and ruggedness of the statistical comparison method, which is essential prior to future implementation in forensic laboratories. In the current work, three sets of structurally similar compounds were investigated: six synthetic cathinones, six synthetic cannabinoids, and six fentanyl analogs. Compounds were prepared at different concentrations and analyzed by gas chromatography-mass spectrometry (GC-MS). Spectra of compounds within each set were statistically compared to evaluate the robustness of the method by taking into account the effect of reduced spectral intensity on the association and discrimination of spectra. Compounds were analyzed on two different GC-MS instruments over several months, with the resulting comparisons used to evaluate the ruggedness of the method.

Preliminary results indicate the potential to successfully associate corresponding compounds while discriminating from structurally similar compounds even as spectral intensity decreases. For example, at a concentration of 1 mg/mL, spectra of pentylone are associated (zero discriminating ions) but discriminated from spectra of dibutylone, eutylone, and propylone, with 7 – 12 ions responsible for discrimination (99.9% confidence level). At lower concentrations, corresponding to 50% and 10%, discrimination is maintained, with 3 – 11 discriminating ions. Initial comparisons also demonstrate the potential to identify reliable discriminating ions, despite differences in spectral intensity and the instrument used to collect spectra. For example, for comparison of dibutylone and pentylone, *m/z* 44, 57, 71, 121, 149, and 150 are common discriminating ions at the three spectral intensity levels investigated and on both instruments tested thus far.

In this presentation, statistical comparison of the synthetic cathinones, cannabinoids, and fentanyl analogs will be presented in more detail and reliable ions for discrimination of structurally similar compounds will be highlighted.

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### Statistical Comparison, Novel Psychoactive Substance, Gas Chromatography/Mass Spectrometry

**B130 The Analytical Challenges of Distinguishing Between Pentylone and Its Positional Isomers**

*Sally Ho, PhD, Drug Enforcement Agency, Chicago, IL*

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**WITHDRAWN**

## **B131 Development of an Expert Algorithm for Substance Identification (EASI) of Fentanyl Analogs using Mass Spectrometry**

*Alexandra Adeoye, BS\*, Department of Forensic and Investigative Sciences, West Virginia University, Morgantown, WV; Glen P. Jackson, PhD, Department of Forensic and Investigative Sciences, West Virginia University, Morgantown, WV and C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV*

**Learning Overview:** At the end of this presentation, attendees will learn how correlated ion abundances within replicate mass spectra can be used to improve confidence in compound identification for fentanyl analogs, especially those sharing structural and spectral similarities.

**Impact Statement:** This presentation will provide the forensic community with a flexible multivariate model that can minimize the risk of false-positive and false-negative identifications and enable confident identifications without the need to analyze contemporaneous drug standards. The goal of the algorithm is to provide sufficient confidence to obviate the need for laboratories to analyze their own drug standards at all.

**Hypothesis:** Our central hypothesis, supported by an extensive database of replicate fentanyl analog spectra, is that relative ion abundances of replicate mass spectra are not independently variable, as has been assumed for more than 70 years, but are in fact highly correlated and anticorrelated. We use the measured correlations that exist between spectra in a series or general linear models to make accurate predictions of ion abundances within spectra.

**Methods/Results:** Most existing search algorithms for electron ionization-mass spectrometry (EI-MS) data use a ‘consensus’ approach when making unknown identifications. These algorithms compare unknown spectra to discrete, fixed spectra of standards in a library. However, the inter-day or inter-laboratory variance in the abundance of each fragment in a spectrum is known to vary by  $\pm 20\%$ , so compounds with somewhat similar EI mass spectra, like many fentanyl analogs, can be difficult to distinguish using only the EI spectral comparison. The multivariate algorithm discussed in this presentation takes a more informed approach and uses an algorithm to effectively interpolate between replicate spectra and provide a continuously variable model of ion abundances for each compound in the database. The model explains most of the variance in replicate mass spectra and enables very confident mass spectral identifications.

A database containing approximately 57,000 replicate mass spectra of 76 fentanyl analogs was compiled from gas chromatography-electron ionization-mass spectrometer (GC/MS) data from 9 different laboratories by extracting every mass spectrum across the eluting chromatographic peaks of interest. The ion abundances for each spectrum were normalized to the base peak in each spectrum. As examples, nine compounds were chosen as known positives (KPs) for model building, and the remaining 75 analogs served as known negatives (KNs). The 20 most abundant ions were extracted, and the abbreviated spectra were randomly divided into training and testing sets. Twenty general linear regression models (GLM) were built for each compound by sequentially using the abundance of each ion as the dependent variable and the abundance of the 19 remaining ions as the independent variables. The models for each compound were then used to predict the ion abundances for various KPs and KNs. The predicted abundances were compared to the measured abundances using various similarity and dissimilarity metrics, like the Pearson product-moment correlation (PPMC) and mean absolute residual. Each metric was then used as a binary classifier to determine the true-positive and false-positive rates over a range of threshold values. These classifications were used to plot a receiver operating characteristic (ROC) curve from which we could calculate the area under the curve (AUC) to determine the strengths of each model.

PPMC values between the measured and predicted spectra of known positives of the selected drug models exceeded 0.9666 for the training and testing sets. Known negatives in the validation set typically had smaller PPMC values than the smallest PPMCs for known positives, resulting in AUCs of 0.999 or better in the ROC plots for binary classification. The residuals in the predictions for the known positives were typically improved by a factor of 3 over the traditional consensus approach. In short, the new algorithm outperforms existing algorithms for accurately identifying fentanyl analogs from their mass spectra.

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### **General Linear Model, Mass Spectrometry, Fentanyl Analogs**



## **B132 Extracted Volatiles from Cannabis Smoke: A Potential Source for the Determination of Detectable Exhalable Breath Components in Users**

*Katherine E. Zink, PhD\**, National Institute of Standards and Technology, Boulder, CO; *Kavita M. Jeerage, PhD*, National Institute of Standards and Technology, Boulder, CO; *Tara M. Lovestead PhD*, National Institute of Standards and Technology, Boulder, CO

**Learning Overview:** This presentation will describe a novel method developed at NIST for the isolation, detection, and characterization of volatile compounds in cannabis smoke that may serve as exogenous markers of recent use in the exhaled breath of cannabis users for law enforcement, public health, and workplace safety.

**Impact Statement:** This project will provide evidence to support further development of a roadside cannabis breathalyzer, an instrument that so far has produced many challenges.

As medical and adult recreational cannabis continues to be legalized across the nation and the world, the need for a rapid roadside detection method to determine recent use is critical. A cannabis breathalyzer has become an intriguing option; in-lieu of blood sampling, the presence of 9 $\Delta$ -tetrahydrocannabinol (9 $\Delta$ -THC) in biological fluids does not provide an indication of last use. Frequent use can result in build-up of 9 $\Delta$ -THC in adipose tissue that may be released over time, even after sustained abstinence.<sup>1</sup> Furthermore, 9 $\Delta$ -THC detection in breath presents challenges due to its low volatility and low concentration.<sup>2</sup>

Cannabis plants have been examined for decades to document the natural products generated by plant metabolism.<sup>3</sup> Most often, this work has been done by solvent extraction of solid plant material, and datasets have generated informative profiles of cannabis compounds. Similarly, volatile molecules from cannabis strains have been profiled for the forensic detection of cannabis by canines.<sup>4</sup> To target detection of cannabis compounds that may be present in breath, this work aims primarily to characterize the volatile components of cannabis that survive combustion in a similar method that users employ while smoking.

An in-house apparatus was designed to burn dried plant samples, collect the volatilized components in the form of smoke, and condense them into both an aqueous fraction and an organic fraction. Flower samples from four hemp strains have been evaluated. The volatiles extracted from these smoke samples were analyzed using liquid chromatography-(LC-UV) and LC mass spectrometry (LC-MS) using a high-resolution instrument, an LTQ Velos Orbitrap (Thermo Fisher). The Orbitrap method measures their intact mass as well as their fragmentation patterns, which provide important structural information. Large datasets were uploaded to the Global Natural Products Social Molecular Networking database (GNPS), an online collection of over 200,000 fragmentation spectra from natural products, including plant compounds. Experimental spectra are compared to annotated spectra in the database both to identify known compounds as well as provide structural information for unknown compounds.

This approach to compound identification has indicated the presence of the cannabinoids 9 $\Delta$ -THC, cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN) in four distinct types of hemp. Additionally, a terpene, nerolidol, has been detected in two of the four strains of hemp. Compounds detected across all samples are candidates for the consideration for the design of a cannabis breathalyzer.

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### **Cannabis, Breath, Smoke**

## B133 Gas Chromatography-Mass Spectrometry of Phencyclidine (PCP) Analogues

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**Learning Overview:** This presentation will report the chromatographic and mass spectrometric data for phencyclidine (PCP) and ten of its analogues on two gas chromatographic columns of different polarity.

**Impact Statement:** This presentation will impact the forensic community by reporting the mass spectral fragmentation patterns and characteristics of PCP and ten of its analogues as well as reporting the retention times (RTs) and linear retention indices (RIs) for these compounds.

**Goal of Project:** The goal of this project was to develop to a gas chromatographic-mass spectrometric method that would separate PCP and ten of its most common analogues and report the retention data (RTs and RIs) and mass spectral information.

Phencyclidine (PCP) and its analogues belong to a class of new psychoactive substances (NPSs) whose prevalence continues to rise. Though these analogues share similar chemical structures to illicit substances, slight modifications can produce unpredictable effects, and limited knowledge of their potential poses a serious threat. Rapid synthesis and distribution of these compounds severely complicates the critical task of analogue identification in forensic laboratories due to lack of reference materials and available standards. Additionally, isomeric distinction may be challenging or even impossible for certain analogues depending on the chosen instrumentation. Contribution to the analytical profile of PCP analogues is necessary so that forensic analysts have the essential resources to make accurate and efficient identifications. Gas chromatography-mass spectrometry (GC-MS) remains a reliable technique for analogue discrimination requiring limited sample preparation and can be optimized for reproducible analysis. In this work, GC-MS was implemented to simultaneously detect and identify PCP and ten of its most common analogues including 3-methoxyphencyclidine (3-MeO PCP), 3-Methoxyeticyclidine (3-MeO PCE), 4-methoxyphencyclidine (4-MeO PCP), diphenidine, methoexetamine (MXE), eticyclidine (PCE), 1-phenyl-N-propyl-cyclohexanamine (PCPr), tenocyclidine (TCP), rolicyclidine (PCPy), and methoxphenidine (MXP). Individual standards were first analyzed to compile their mass spectra, interpret and elucidate their fragmentation patterns, and confirm their identity. A mixture of these compounds was then prepared for analysis on two different column stationary phases of varying polarity, HP-1 MS and Rxi-5Sil MS. Both columns had the same configuration (30 m x 25 mm x 0.25  $\mu$ m). Split injection (25:1) was used for all analyses. Separation of up to ten standards was achieved, though baseline resolution of TCP and PCP was only attained on one column, the non-polar 100% dimethylpolysiloxane (HP-1 MS). Retention data including retention times and calculated linear retention indices showed good reproducibility ( $CV \leq 0.1\%$ ) over the course of ten days for each column. Averages of these values were reported with their corresponding relative standard deviation for each analogue. Of the standards, 4-methoxyphencyclidine (4-MeO PCP) experienced degradation appearing to decrease with lowered inlet temperatures. The artifact peak could not be completely eliminated even with decreased inlet temperatures. The base peak for 4-MeO PCP tended to shift, likely influenced by the abundance of the degradation, and leading to varied ion intensities in the mass spectrum. While general fragmentation patterns were followed, all analogues produced distinct mass spectra capable of distinguishing all compounds studied. Supplementation of comprehensive data such as elucidation of mass spectral fragments and retention data (RT and RI) for a large set of PCP analogues will help bridge the gap between drug synthesis and identification by offering comparable reference material to aid in more productive forensic drug analysis and combat the challenges of NPS identification.

### GC/MS, PCP, Retention Indices

## B134 HPTLC Separation of Tryptamine Based Hallucinogens

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**Learning Overview:** This presentation will report the evaluation of seven thin-layer chromatography (TLC) mobile phase systems to separate 12 natural and synthetic hallucinogenic tryptamines using high-performance thin-layer chromatography (HPTLC).

**Impact Statement:** This presentation will impact the forensic community by demonstrating that HPTLC can be a useful and viable alternative tool in the seized-drug analysis of natural and synthetic hallucinogenic tryptamines.

**Goal of Project:** The goal of this project was to evaluate TLC mobile phase systems to determine the optimum conditions for the separation of 12 natural and synthetic hallucinogenic tryptamines using HPTLC.

Traditional thin-layer chromatography (TLC) has been one of the analytical techniques used for the analysis of common hallucinogenic tryptamines for many years. However, novel psychoactive substances (NPSs) are constantly being synthesized and these compounds need to be incorporated into existing laboratory procedures. Using traditional TLC does not always provide a satisfactory level of sensitivity, resolution, or documentation to analyze all of these compounds, many of which may be closely related structural isomers/analogs. In this study high-performance thin-layer chromatography (HPTLC) was used to separate 12 tryptamine hallucinogens, including the commonly analyzed psilocybin, psilocin, and bufotenine. The other nine tryptamines included in the study were 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT), 5-methoxy- $\alpha$ -methyltryptamine (5-MeO-AMT), 5-methoxy-N,N-diallyltryptamine (5-MeO-DALT), 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT), 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT), N,N-diethyltryptamine (DET), N,N-dipropyltryptamine (DPT), N,N-dimethyltryptamine (DMT), and 12-methoxy-ibogamine (ibogaine). The visualization reagent p-dimethylaminobenzaldehyde (p-DMAB) was sprayed on each plate to derivatize the compounds so they could be more easily detected by the naked eye. Seven different mobile phase systems which included 100:1.5 methanol/ammonium hydroxide, 2:1:1 n-butanol/water/glacial acetic acid, 75:15:10 cyclohexane/toluene/diethylamine, 2:1 n-propanol/5% ammonium hydroxide, 4:1 chloroform/methanol, 75:10:15 methanol/glacial acetic acid/water, and 100:1.5 methanol/glacial acetic acid were investigated to determine which produced optimal separation for all the compounds. Six of these mobile phases were based on previous literature and one was developed from ratios and solvents of previously tested mobile phases. The mobile phases evaluated were considered successful based upon whether the compounds interacted with the mobile phase and migrated up the plate ( $R_F > 0.1$ ), the number of pairs of compounds that could be resolved from each other ( $R_s > 1$ ), reproducibility ( $CV < 10\%$ ), and if the visualization reagent resulted in a visible color reaction. Out of the seven mobile phases, 100:1.5 methanol/ammonium hydroxide (mobile phase 1), 2:1:1 n-butanol/water/glacial acetic acid (mobile phase 2), and 100:1.5 methanol/glacial acetic acid (mobile phase 7) produced the most successful results. Mobile phases 1, 2, and 7 were able to provide  $R_F$  values that spanned the majority of the plate. These mobile phases also provided good resolution between almost all of the compounds. Although mobile phase 1 provided resolution between some of the tryptamines, the reproducibility of the  $R_F$  values was unsatisfactory compared to mobile phases 2 and 7. Taking both resolution and color into consideration, 66 pairs of tryptamines were compared. Mobile phase 2 was able to differentiate between 56 out of 66 possible comparisons between compounds and mobile phase 7 can differentiate between 60 pairs of compounds. Using either of these mobile phases in conjunction with p-DMAB shows promising results when trying to distinguish between psilocin, psilocybin, bufotenine, and 9 other tryptamine compounds. The results suggest that HPTLC is a suitable method for separating common tryptamine hallucinogens while incorporating adequate sensitivity, good resolution, along with providing proper documentation, and ecofriendly science.

### HPTLC, Tryptamines, Hallucinogens

## B135 Interpreting DNA Mixtures With Extreme Allele Overlap

Tim Kalafut, PhD\*, Sam Houston State University, Huntsville, TX; Jo-Anne Bright, PhD, Institute of Environmental Science and Research, Mt Albert, Auckland, New Zealand; Duncan Taylor, PhD, Forensic Science SA, Adelaide, SA, Australia

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the interpretation process and outcomes for mixtures where non-donors (H2 True) have a high level of allelic overlap with the true donors (H1 True).

**Impact Statement:** This presentation will impact the forensic science community by discussing appropriate ways to approach mixtures where there is a concern about the presence of non-donors that are related to donors.

There is considerable interest in the effect of relatives on the interpretation of mixed forensic DNA samples using probabilistic genotyping. Recent examples of interest are found in the NISTIR 8351 draft report and a promised report by the Brooklyn Defender Services.<sup>1,2</sup> There are two situations where it would be helpful to differentiate: 1) where a relative of a true donor or donors is falsely considered to be a donor to the mixture, and 2) where the true donors are themselves related.

It is known that relatives of true donors have a higher rate of adventitious support than unrelated false donors. It is also known that the high degree of allelic overlap by relatedness of the true donors can make assignment of number of contributors (NoC) and deconvolution difficult. This was noted at least 30 years ago by Evett, yet there is a call “to bring these troubling analytical findings out of the shadows and issue a clear public warning about the unconscionable risk of falsely including a non-contributor relative in a DNA analysis.”<sup>2,3</sup>

Theory suggests that mixtures of two relatives (dyads), although showing a degree of allelic overlap, should not present extreme difficulties. Lin et al. showed that mixtures of two parents and a child did present such difficulties.<sup>4</sup> We hypothesize that no dyads should present extreme difficulties to assignment of NoC or deconvolution but that some triads will do so. Specifically, we hypothesize that PPC, PCC, SSS mixtures will present difficulty.

We present here the results of 177 H1 true, 42,419 H2 true unrelated, and 14,053 H2 true related tests on in-vitro constructed mixtures and 1,150 H1, 128,850 H2 true unrelated, and 50,300 H2 true related tests on in-silico constructed mixtures of dyads and triads. No dyads presented difficulty for NoC assignment or deconvolution. We detected no instances of false exclusion or support in our in-vitro set. The in-silico set showed a considerable rate of false support especially for the related non-donors. The likelihood ratios (LRs) for this false support were of considerable magnitude. There was a considerable difference in template with the in-vitro samples having much higher template.

All balanced triads in our in-vitro set presented difficulty in the assignment of NoC and deconvolution. The most likely outcome of an uninformed examination of such a triad is an incorrect assignment of NoC = 2. This provokes false exclusion but does not appear to provoke false support. When the balanced triads were examined as NoC = 3, the deconvolution struggled to find a third contributor. This is expected, as three equally contributing full siblings look like a 2:1 mixture of unrelated persons. Due to an incorrectly low assigned proportion for a third donor, both false exclusionary LR and false support for related and unrelated profiles were obtained. When allele overlap to the mixture is considered for false related inclusions, the LR are often not intuitive for such high allele sharing.

The work described here explored ways to improve the deconvolution and minimize false exclusions and false inclusionary support for unrelated and related donors. The effects of conditioning profiles and use of pre-determined donor proportions was examined (Mx prior). Together, these strategies show an improvement in the LR for donors and decrease false support for both related and unrelated nondonors. This shows that the deconvolution and LR calculation should not be done in a vacuum. Relevant case information is necessary to minimize both false exclusions and false inclusionary support. It is recommended that references from all relatives, especially first order relatives be tested if there is concern about close relatives in a case.

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### Overlap, Mixtures, Relatedness

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## **B136 The Effect of Pull-Up on the Allele Peak Heights and the Weights of Genotypes for Minor Donors**

*Damani Johnson, BS\*, Sam Houston State University, Huntsville, TX; Tim Kalafut, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how spectral pull-up can affect low-level data peaks and the subsequent genotype weights used to calculate a probabilistic likelihood ratios (LR).

**Impact Statement:** This presentation will impact the forensic science community by discussing a method that has the potential to give more weight to the ground truth genotypes of trace donors in a mixture.

Capillary Electrophoresis of fluorescently labeled PCR products is the most common form of forensic DNA analysis in crime laboratories today. A fluorescent tag is applied to the PCR product, and after traveling through the capillary, a laser is used to excite the tag and a camera reads the signal that is produced and measured as relative fluorescence units (rfu). One issue that needs to be addressed is that these tags do not fluoresce at a single wavelength, and there is spectral overlap between them. An attempt to compensate for this is done by the use of a spectral calibration where an algorithm is applied that is intended to correct for the overlap.

When the fluorescence signal is strong, such as for a sample with a high amount of DNA, it is common to reach the limit of this spectral correction. This results in a signal from a PCR product in one channel (primary pull-up peak) causing so-called “pull-up” peaks in one of the other dye channels. The primary pull-up peaks in the dye channel of the PCR product and the resultant pull-up peaks in the affected channel are closely correlated in time. If the pull-up is strong enough, these artifactual pull-up peaks can be sized like an actual PCR product signal, as they can be above the analytical threshold. Often the pull-up peaks do not correspond to any peak on the ladder and are relatively easy to identify as artifactual in nature. Occasionally, due to the lay out of the loci in the PCR kit, the pull-up peaks can be “on ladder” and show all the characteristics of an actual PCR product peak.

This can complicate the deconvolution of major/minor mixtures if the height of a minor donor peak is taller than expected due to the summation of the actual minor donor PCR product rfu and the additional rfu from the pull-up effect. If a pull-up peak is added to a stutter peak, it can result in a stutter PCR product to be labeled as an allele because of the additional pull-up rfu. If an unshared peak of a minor donor is enhanced by the pull-up effect, the minor genotype might appear to be a homozygote, when really the sister allele is masked by an allele of the major donor.

The OSIRIS software program (NIH) has some ability to correct for pull-up when a pattern is recognized across channels. ArmedXpert™ (NicheVision, LLC) has the ability to pull the corrected value out of the data, and then use that value in the preparation of data tables. This allows for a corrected peak height to be used in STRmix™ (ESR, Ltd) for probabilistic genotyping. We prepared mixtures of major and minor donors using the Investigator 24plex QS kit (QIAGEN) where primary pull-up peaks from the major donor correspond to actual peaks from the minor donor, resulting in a higher rfu value. We investigated if using the corrected rfu value for the minor peak (subtracting out the pull-up contribution) gave more weight to the known genotype of the minor donor and made the LR more informative (larger) for those affected loci.

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### **Pull-Up Peaks, Genotype Weights, Corrected RFU**

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## B137 The Detection and Analysis of DNA Mixtures With the MiSeq® FGx™

*Rachel Houston, PhD\*, Sam Houston State University, Huntsville, TX; Timothy Kalafut, PhD, Sam Houston State University, Huntsville, TX; Ryan Gutierrez, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** This presentation will address the advantages and disadvantages of implementing Next Generation Sequencing (NGS) to detect and deconvolute DNA mixtures.

**Impact Statement:** Continued research and evaluation of NGS platforms for forensic casework will promote further adoption of this technology in operational crime labs.

It is often necessary to process samples that contain biological material from more than one donor in forensic cases. This can include evidence from sources such as sexual assault kits to property crime. Mixed samples are variable and can have limited input samples and various combinations from major and minor donors. To successfully process these samples, it is necessary to identify the presence of low-level mixture alleles as well as the number of mixture contributors before further interpretation.

NGS may help analysts by providing distinct advantages in allele recovery leading to better estimations for the number of contributors (NoC), including multiple male and female donors. In addition, NGS may provide more confidence in relation to NoD due to the potential for different isoalleles between donors. This research analyzed mock forensic and mixture samples with the ForenSeq™ DNA Signature Prep kit Primer Set A for sequencing on the MiSeq FGx®. In addition, all samples were compared with complimentary amplification using the GlobalFiler™ Kit for capillary electrophoresis. Metrics tested for both chemistries included concordance, limits of detection, and mixture analysis. Data analysis for mixture samples was completed with the MixtureAce™ plug-in and ArmedXpert™ software. NGS offered distinct advantages in determining the numbers of male contributors, limits of detection, and iso-allele heterozygosity but suffered from increased variability in stutter and peak height ratios compared to capillary electrophoresis.

Any new forensic techniques that are implemented must be able to handle the challenges of mixed forensic samples. NGS offers advantages over capillary electrophoresis in amplicon multiplexing and degraded sample analysis. However, advantages with mixture samples will rely heavily on the advancement of user-friendly analysis software to supplant current probabilistic genotyping pipelines that are available for use with capillary electrophoresis data.

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### Next Generation Sequencing (NGS), Mixture Analysis, Isoalleles

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## **B138 Enhanced Mixture Interpretation With Macrohaplotypes Based on Long-Read DNA Sequencing**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the latest development on long-read DNA sequencing technologies, how the macrohaplotypes are designed based on the long-read data, and how these macrohaplotypes can be used to enhance DNA mixture interpretation.

**Impact Statement:** This presentation will impact the forensic science community by providing a new set of forensic markers that substantially outperform CODIS STRs for mixture interpretation, particularly for mixtures with a high number of contributors.

Deconvoluting mixture samples is one of the most challenging problems confronting DNA forensic laboratories. Efforts have been made to provide solutions regarding mixture interpretation. The probabilistic interpretation of Short Tandem Repeat (STR) profiles has increased the number of complex mixtures that can be analyzed. A portion of complex mixture profiles, particularly for mixtures with a high number of contributors, are still being deemed uninterpretable. Novel forensic markers, such as Single Nucleotide Variants (SNV) and microhaplotypes, also have been proposed to allow for better mixture deconvolution. However, these markers have both a lower discrimination power compared with STRs and are not compatible with CODIS or other national DNA databanks worldwide. The short-read sequencing (SRS) technologies can facilitate mixture interpretation by identifying intra-allelic variations within STRs. Unfortunately, the short size of the amplicons containing STR markers and sequence reads limit the alleles that can be attained per STR. The latest long-read sequencing (LRS) technologies can overcome this limitation in some samples in which larger DNA fragments (including both STRs and SNVs) with definitive phasing are available.

Based on the LRS technologies, this study developed a novel CODIS compatible forensic marker, called a macrohaplotype, which combines a CODIS STR and flanking variants to offer extremely high number of haplotypes and hence very high discrimination power per marker. The macrohaplotype will substantially improve mixture interpretation capabilities. Based on publicly accessible data, a panel of 20 macrohaplotypes with sizes of ~8k bp and the maximum high discrimination powers were designed. The statistical evaluation demonstrates that these macrohaplotypes substantially outperform CODIS STRs for mixture interpretation, particularly for mixtures with a high number of contributors, as well as other forensic applications. Based on these results, efforts should be undertaken to build a complete workflow, both wet-lab and bioinformatics, to precisely call the variants and generate the macrohaplotypes based on the LRS technologies.

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### **Macrohaplotype, Long-Read DNA Sequencing, DNA Mixture**

## **B139 Results From a Probabilistic Genotyping Software for the Continuous Interpretation of Next Generation Sequencing (NGS) Autosomal Short Tandem Repeat (aSTR) Mixtures**

Kevin Cheng, MSc\*, Institute of Environmental Science and Research (ESR), Auckland, Auckland, New Zealand and The University of Auckland, Auckland, New Zealand; Jo-Anne Bright, PhD, Institute of Environmental Science and Research (ESR), Auckland, New Zealand; James Curran, PhD, The University of Auckland, Auckland, New Zealand; John Buckleton, DS, Institute of Environmental Science and Research (ESR), Auckland, Auckland, New Zealand and The University of Auckland, Auckland, New Zealand

**Learning Overview:** The aim of this presentation is to show the deconvolution results of NGS aSTR mixtures using published biological models implemented into a prototype probabilistic genotyping software.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a possible probabilistic genotyping solution for the interpretation of mixtures generated using sequencing technology.

There has been increasing interest from forensic laboratories to evaluate the use of next-generation sequencing (NGS) technologies or massive parallel sequencing (MPS) technologies. For example, the use of sequencing technology for genetic genealogy, missing-persons identification, or paternity testing.<sup>1</sup> One particular area of interest is the use of this technology to interpret autosomal short tandem repeats (aSTRs) in forensic mixtures. This is because, unlike contemporary capillary-electrophoresis (CE) methodologies for the analysis of aSTRs, NGS can detect sequence variations in a stretch of STRs. Several publications have demonstrated that this sequence-level information can increase the power of discrimination in single-source profiles.

Over the past few years, there have also been a number of publications describing the behavior of STRs in NGS-DNA profiles including publications around potential quantitative models to describe an expected NGS-DNA profile.<sup>2-22</sup> This increasing body of knowledge has facilitated the development of probabilistic genotyping solutions for the interpretation of aSTR mixtures generated using sequencing technologies. Using the biological models described by Cheng et al., models that were adapted from Bright et al. and Vilsen et al., a prototype probabilistic genotyping solution was created.<sup>10,22,23</sup>

This presentation will present the results of the continuous interpretation of aSTR mixtures that were generated using a sequencing technology with this prototype probabilistic genotyping solution. This will include the assignment of likelihood ratios (LRs) to known donors and non-donors to more than one-hundred DNA profiles that were experimentally designed to contain up to four-contributors. The results demonstrate the behavior of the LRs, the changes in the LR when a known donor to the mixture is assumed, and the effect of under- or over-assigning the number of contributors to a mixture. The presentation will also describe some of the limitations in this prototype solution such as degradation and the assignment of the number of contributors, and possible solutions to address these limitations.

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## NGS, Probabilistic Genotyping, Mixture Interpretation

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### **B140 The Recovery of Probative DNA Information From Complex Mixtures Involving First-Degree Relatives or Marginally Detectable Minor Donors Using Direct Single Cell Subsampling (DSCS) and Probabilistic Genotyping (PG)**

*Kaitlin Huffman, MS\*, University of Central Florida, Orlando, FL; Erin Hanson, PhD, University of Central Florida, Orlando, FL; Jack Ballantyne, PhD, University of Central Florida, Orlando, FL*

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**Learning Overview:** Attendees will learn of a DNA mixture deconvolution tool that relies on subsampling of individual or few cells by physical capture, subsequent high sensitivity DNA typing and quantitative computer interpretation to extricate probative single source and mixed DNA genotypes.

**Impact Statement:** The DSCS approach could permit forensic scientists to analyze and recover probative evidentiary information from complex mixtures not only from those with excessive overlapping alleles such as those seen with related individuals, but also from marginally detectable minor donors.

DNA mixtures are one of the more difficult sources of biological evidence to deconvolute and interpret. This is particularly troublesome as current STR typing kits have increased sensitivity increasing the prevalence in which DNA mixtures are seen. Probabilistic genotyping (PG) has greatly aided in mixture analysis. However, even with PG, standard bulk mixture approaches do not always result in probative results.

One such instance is a mixture in which there is an extreme minor contributor resulting in a virtually undetectable donor even when large quantities of DNA are present. An example of this is a 2-person mixture where a weight ratio of ~1:50 is seen. Furthermore, mixtures with significant allele overlap can lead to inaccuracy in determining the number of donors within a mixture potentially resulting in missed contributors. This can notably be seen with mixtures comprising of related individuals which also increases the probability of false inclusions from other non-contributor relatives.

To aid in mixture deconvolution and genotype information loss seen in these especially complex mixture scenarios, a simple micromanipulation technique referred to as Direct Single Cell Subsampling (DSCS) has been applied. DSCS allows for physical separation of subsets of cells (typically 1-5 cells) from mixtures prior to DNA typing. Low template DNA techniques (LTDNA) and probabilistic genotyping validated for use with few cells was applied. PG software systems, STRmix™ and EuroForMix, were utilized to compare the information loss (in bans) of the standard 'bulk' mixtures to that of the contributor single source reference profiles. Comparison to the analyzed DSCS samples obtained indicated instances in which the DSCS method resulted in a substantial gain of probative information.

The feasibility of this approach will be presented firstly using a complex two-person 1:51 buccal cell mixture in which one donor is nearly undetectable. By taking multiple 5-cell samplings, the probability of obtaining genetic information for that minor donor is increased. Instances in which the minor donor is retrieved results in new mini mixtures with a 1:4 weight ratio and a significant increase in LRs. This approach will further be applied to mixtures comprised of donors, some of whom are first degree relatives.

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#### **Mixtures, Micromanipulation, Single Cells**

## B141 Automated Familial Search Using a Probabilistic Genotype Database

*Matthew Legler, BS, Cybergenetics, Pittsburgh, PA; Kevin Miller, PhD, Hamilton Robotics, Reno, NV; Mark Perlin, PhD, MD\*, Cybergenetics, Pittsburgh, PA; Garrett Sugimoto, MS, Kern Regional Crime Laboratory, Bakersfield, CA*

**Learning Overview:** To evaluate a workflow for the automatic development and comparison of probabilistic genotypes from complex evidence for the purpose of inferring a perpetrator genotype that may not be previously known.

**Impact Statement:** Forensic laboratories may use local DNA databases to develop automated familial searching workflows that yield impactful increases in investigative leads for law enforcement.

Familial searching can find suspects by searching a DNA database that contains their relatives. Genotypes from people who contributed their DNA to crime scene evidence are compared with reference profiles in the DNA database. Even when the contributor genotypes are not in the database, genetic similarities to contributor relatives who are in the database can help find investigative leads.

Earlier familial search strategies were based upon partial allele matches and simple allele inclusion statistics. Cybergenetics TrueAllele® technology provides a more sophisticated approach using information-preserving probabilistic genotypes, and likelihood ratio (LR) information.

The TrueAllele database automatically generates probabilistic familial genotypes from reference genotypes, using them for automated comparison with probabilistic genotypes from complex evidence. LR match statistics are calculated automatically. This general computer method allows any DNA evidence (e.g., mixtures of up to ten contributors) to be easily compared with automatically inferred familial genotypes.

The Kern Regional Crime Laboratory (KRCL) conducted three validation studies prior to deploying their information driven automated TrueAllele familial search system.

An *Accuracy Study* demonstrated the software can provide informative match statistics to evidence items when compared to inferred kinship genotypes from relatives of true contributors. DNA profiles from 26 individuals from four different family trees were typed and included in the accuracy study. There were 676 pairwise comparisons made for each assumed genotype group (PCH, SIB and HAS) for a total of more than 2,000 comparisons. The evidence to parent/child (EVI-PCH) and evidence to sibling (EVI-SIB) searches were shown to be informative.

A *Sensitivity and Specificity Study* examined LR comparison of PCH and SIB inferred genotypes when the mixture weight of the true contributor's DNA varied. Thirty-one single source profiles and 36 mixture samples (2 to 5 contributors) were compared to the inferred genotypes of 44 single source known reference profiles. The EVI-PCH searches were more specific for inferred genotypes of closely related relatives of known contributors. The EVI-SIB searches were more sensitive for inferred genotypes of more distant relatives of known contributors. The EVI-PCH false positive rate was ~0.02% (1/6512), with  $\log(\text{LR}) = 2.117$  ban. The EVI-SIB false positive rate was ~0.25% (16/6512), having  $\log(\text{LR})$  values from ~2.0 to 4.5 ban.

A *Known and Nonprobative Evidence Study* compared kinship inferred genotypes in a larger database comparable to the KRCL's local DNA database. This study included known reference samples from known matches, used to compare to EVI profiles in the laboratory's local database of over 5,000 profiles. The EVI-PCH and EVI-SIB searches accurately matched to the inferred genotypes of known contributors, as well as the inferred genotypes of the true contributor's son. The EVI-PCH comparisons were more specific than EVI-SIB, resulting in fewer false positives. EVI-PCH search did not result in any match scores above 1,000, while EVI-SIB search gave 25 false positives with match scores greater than 1,000. The false positive rate (for a  $\log(\text{LR}) \geq 2$ ) was ~.0001% for EVI-PCH searches, and ~.0006% for EVI-SIB searches.

Familial search is a powerful tool for finding investigative leads from DNA evidence when other avenues have not produced suspects. The KRCL has conducted validation studies and developed protocols to bring automated TrueAllele familial searching into routine forensic practice. This forensic science advance can make more identification information available to criminal justice by reusing data from existing DNA evidence.

### Validation, Familial Genotype, Probabilistic Genotyping

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## B142 Using DNA Mixtures for Relationship Inference and Missing Persons Identification

*Maarten Kruijver, PhD\*, Institute of Environmental Science and Research, Auckland, Solid, New Zealand*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how probabilistic genotyping can aid missing persons identifications.

**Impact Statement:** This presentation will impact the forensic science community by explaining novel methods at the intersection of probabilistic genotyping and relationship inference.

Recent theoretical advances in probabilistic genotyping enable relationship inference when one or more of the observed DNA profiles are mixed or low-level.<sup>1</sup> This bridges the fields of probabilistic genotyping and kinship testing unlocking new applications in both fields. This presentation focuses on how probabilistic genotyping can be used to draw inferences about blood relationships and discusses applications in missing persons identification.

In missing person cases, DNA may be used to establish a link between unidentified remains and a missing person. When a preliminary link is not already available from non-genetic evidence, a DNA database search can be used to compare a reference of the missing person to profiles obtained from unidentified remains. These cases can be complicated if a reference profile from the missing person is not (yet) available.

Exploiting theory from probabilistic genotyping, the presentation explains how a list of weighted genotypes can be obtained for the missing person based on other relevant DNA profiles. These profiles may be mixed DNA profiles to which the missing person is a contributor but also include any profiles to which close relatives of the missing person are a contributor. These weighted genotypes can be used for database searching combining all available DNA data just like how mixture deconvolutions can be used for database searching in criminal casework.<sup>2</sup> The use of weighted genotypes makes it possible to compare large numbers of profiles at little computational cost. With modern multiplexes, the power to identify a person based on DNA of their relatives can be substantial. It is explained how case-specific false positive rates and true positive rates can be estimated using simulation. The simulation results can be used to evaluate whether the DNA data is sufficiently informative for identifications or that further data is needed.

The presentation emphasizes how theory from probabilistic genotyping has further applications besides criminal casework.

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**Probabilistic Genotyping, Relationship Testing, DNA Mixture**

## B143 “Essentially, All Models Are Wrong, but Some Are Useful”—A Comparison of Likelihood Ratios Obtained From EuroForMix and STRmix™

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**Learning Overview:** The aim of this presentation is to show the comparison of likelihood ratios obtained between two probabilistic genotyping software. Similarities and differences between probabilistic genotyping software were assessed with single-source profiles and 129 mixtures.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that even though there are differences between the software both can be useful in assigning a likelihood ratio (LR). The inclusion of two sets of developers as collaborators and developers within this study should alleviate the concern that the work will be biased towards a single model and provide an in-depth understanding of the two software.

The British statistician, George Box, has been famously quoted as saying “Essentially, all models are wrong, but some are useful.” By saying that “all models are wrong” is to say that every model makes some fundamental assumptions about reality, no model can ever hope to cover all the intricacies of a real-world system.<sup>1</sup> This is applicable to all probabilistic genotyping (PG) software used to assist with the interpretation of forensic DNA profiles, where there are many modelling assumptions made about the interpretation of forensic DNA profiles. With a good understanding of each software, the differences arising from these assumptions can be predicted; and in some cases, software options or workarounds allow these differences to be minimized.

Although making these assumptions, or simplifications of reality, means that the models are “wrong” they can be very useful for better understanding of what is being modelled and predicting the outcome given certain inputs. The use of models within PG software allows forensic practitioners to evaluate DNA profiles and assign LRs to a pair of propositions. The question is then whether the LR from different PG software are “equally reliable” or “equally useful.”

It is known that different models implemented in different software can produce divergent results. This thinking is reflected in the 2016 PCAST (President’s Council of Advisors on Science and Technology) report, where the authors called for (amongst other things) an investigation into “Under what circumstances – and why – does the method produce results (random inclusion probabilities) that differ substantially from those produced by other methods?”<sup>2</sup> This presentation aims to summarize the results from a comparison of LRs obtained from the interpretation of more than 120 PROVEDIt profiles using two probabilistic genotyping software – EuroForMix and STRmix™.<sup>3,4</sup> The results from both software show similar LR behavior, suggesting that even though the models in the two software are different, both can be useful in assigning an LR. Any difference in the assigned LR between the software were investigated to try and identify the driving factor(s). This body of work was carried out concurrently with the independent comparison work published by Riman et al. and reinforces their findings.<sup>5</sup>

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### Probabilistic Genotyping, Mixture Interpretation, STR

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## B144 Peeling Away Uncertainty: A Probabilistic Approach to DNA Mixture Deconvolution

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**Learning Overview:** After attending this presentation, attendees will understand how genotype conditioning improves DNA mixture separation, decreases genotype uncertainty, and increases DNA match information.

**Impact Statement:** This presentation impacts the forensic science community by describing how the genotype conditioning method increases the identification information that can be obtained from complex DNA mixtures.

Complex mixtures contain DNA from multiple contributors in differing amounts. Unlike older manual methods, probabilistic genotyping (PG) can use all of the peak data to separate the DNA contributors out of these mixtures into their component genotypes. Once the genotypes are inferred, they are compared to reference genotypes (relative to population allele frequencies) to calculate likelihood ratio (LR) match statistics. These statistics can be inclusionary or exclusionary.

Genotype comparison LR values scale with inferred genotype certainty, where more definite genotypes show higher probability allele pair possibilities. More definite genotypes lead to larger (i.e., more informative) match statistics, whether inclusionary or exclusionary.

Uncertainty in the inferred genotypes – seen as a diffuse allele pair probability distribution – reduces this match information. Genotype uncertainty can be due to incomplete separation of the DNA contributors, low DNA amounts, or biological relatedness between contributors. More genotype uncertainty leads to less LR information. Therefore, identification information can be increased by reducing genotype uncertainty.

Genotype conditioning, or ‘peeling’, assumes a statistically established contributor genotype in genotype inference. This conditioning ‘locks down’ a contributor genotype, focusing the inference on the other unknown contributor genotypes. Conditioning can improve the genotype mixture separation, reducing uncertainty in the other genotypes, and increasing match information.

This study demonstrates the utility of genotype conditioning using the TrueAllele® PG system. Mixture samples (genotypes and their amounts) were randomly designed. Two, three, four and five DNA contributors were examined. Initial computer processing established the most informative DNA contributors. Subsequent computer runs used these statistically established genotypes to reduce uncertainty in the remaining unknown genotypes. Multiple rounds of processing ‘peeled away’ the unknown contributor genotypes until one remained. Assuming all contributor genotypes as known gave highly accurate contributor mixture weights.

Inferred genotypes were compared with 70 references to calculate LR match statistics. The log(LR) information increased linearly with contributor DNA amount, until reaching a maximal information plateau for major contributors.

Genotype conditioning improved separation for overlapping contributors, overall doubling their match information. For example, a five-contributor mixture contained two 11% components. Initially, the LRs for these overlapping contributors were in the thousands. Peeling these contributors away from the major contributors increased their LR values into the billions, while peeling the contributors away from each other increased the LRs to almost a quintillion.

An adjudicated quintuple homicide case demonstrated how genotype conditioning works to support criminal justice. Prosecutors used a probative five-person mixture sample, taken from a spot of blood on a garage floor, to establish victim family members’ presence at a suspect’s home. The forensic question was to determine which family members left their DNA in the blood spot. While initial computer results were informative, more analysis could be done.

Through multiple rounds of genotype conditioning, TrueAllele separated the garage floor mixture into component family member genotypes. Peeling away DNA contributors statistically showed the presence of both parents, their children, and another relative. Conditioning also showed that another family member, whose DNA was not in the evidence, was statistically excluded from the mixture. While family allele sharing had initially indicated an inclusionary match statistic, genotype peeling factored away genotypes of relatives to show her DNA was not in the mixture.

This presentation demonstrates both the laboratory and real-world utility of genotype conditioning, and how the method reduces DNA mixture uncertainty by increasing information.

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### Genotype Conditioning, Peeling, Mixture Deconvolution

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### **B145 How Forensic Genetic Genealogy (FGG) Is Supporting the Combined DNA Index System (CODIS) and the Criminal Justice System**

*Colleen Fitzpatrick PhD\*, Identifinders International LLC, Fountain Valley, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how Forensic Genetic Genealogy (FGG) supports conventional means of human identification through CODIS.

**Impact Statement:** This presentation will impact the forensic science community by describing the overlap and compatibility of FGG identification methods with those of CODIS.

CODIS has been the backbone of human identification for 30 years and will continue to be the legal form of human identification for the foreseeable future. In the meantime, Forensic Genetic Genealogy (FGG) has moved from a miracle cure for the common cold case to its recognition as a practical tool that can be used to generate investigative leads.

Due to the broader reach of crowd-sourced data, FGG is helping to identify perpetrators of decades-old cold cases that have not achieved CODIS hits; unlike CODIS, FGG does not require an offender to be in the database. Consequently, FGG is often the only means to identify offenders who do not otherwise have a criminal record, as well as offenders who died before CODIS was in place.

Even if an individual has committed other offenses, however, there are still reasons why he may not be in CODIS yet may be identified through FGG. States vary widely on the type of crimes for which DNA collection is required and when that collection can take place; only a few states practice familial searching. There have also been lapses in the criminal justice system where “lawfully owed DNA” should have been collected but was not.

FGG benefit CODIS in other ways. FGG has shown useful to provide exclusions via target testing and information on ethno-ancestry and phenotype, and to identify witnesses using crime scene DNA that is ineligible for CODIS upload. It has also been used successfully to identify unidentified remains where family members were not in the CODIS Missing Persons Database.

There are additional relevant comparisons that can be made between genetic genealogy in general, FGG in particular, and CODIS, that draw a larger picture of the public’s attitude towards the criminal justice system. While the expansion of DNA collection laws and the use of familial searching has caused push-back on the side of privacy versus public safety, commercial Direct-to-Consumer DNA testing company databases have grown to be enormous in size, backed only by testing company privacy policies and terms of service agreements that can change unilaterally and without notice. Ironically, CODIS has approximately 20 million entries from convicted offenders and arrestees while Direct-to-Consumer (DTC) DNA testing databases have topped about 40 million entries. Of the approximately 1.4M GEDmatch users, only about 400,000 have opted-in to allow law enforcement use of their data.

This presentation explores how FGG supports CODIS and the criminal justice system, through a draft survey of FGG casework that has provided insight into the synergy between the two methodologies. The presentation briefly mentions issues with the use of crowd-sourced DTC consumer DNA data for forensic applications, and ends with a discussion on what the future holds for FGG as it continues to develop as a game-changing investigative tool.

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**FGG, CODIS, DNA**

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## **B146 Development and Implementation of Forensic Genealogy Casework Acceptance Criteria and Testing Guidelines and Their Impact on Developing Valuable High-Density SNP Datafiles.**

*Robert Bever, PhD\*, Bode Technology, Lorton, VA; Teresa Vreeland, BS, Bode Technology, Lorton, VA*

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**Learning Overview:** Attendees will learn how to develop casework acceptance criteria for forensic genealogy casework and how the acceptance criteria impact the ability to generate valuable high-density SNP datafiles.

**Impact Statement:** The presentation will impact the forensic community by demonstrating the value of establishing acceptance criteria for forensic genealogy casework. It will also provide data that demonstrates that low quantity and poor-quality DNA can generate valuable SNP datafiles that can be utilized in forensic genealogy investigations.

Developing a casework acceptance criteria is particularly important for forensic genetic genealogy (FGG) casework due to the age, low quality, and low amount of the evidentiary material associated with cold casework evidence. Therefore, to efficiently process criminal casework and conserve the evidence samples, an acceptance criteria was developed for FGG casework to be processed via micro-array or genomic sequence analysis. The acceptance criteria were established associated with the amount of DNA. Varying amounts of DNA (100 ng to 0.25 ng) extracted from each sample type were analyzed using either the Illumina GSA V2 chip or Illumina NovaSeq 6000 Genome Sequencing workflow to produce high density SNP datafiles to search for matches in the third-party databases. The results indicate that valuable high-density SNP datafiles with call rates > 60% generated from either microarray analysis or genomic sequence analysis can be produced from DNA templates as low as 0.25 ng from blood and 2 ng from sperm. Genomic testing guidelines were developed based on the Degradation Index (DI), to decide whether to process the evidentiary DNA using micro-array analysis or genome sequencing. DNA was degraded from a DI of 1.0 to >20 and then processed via micro-array or genome sequence analysis. DNA degraded to DI of > 6.0 and analyzed via microarrays produced high density SNP files with failed call rates 60% when the datafile was generated by genome sequencing; therefore, the Degradation Index of 6.0 was used as the testing guideline metric to decide whether to process a sample by micro-array or genome sequencing. The acceptance criteria and testing guidelines were further validated by demonstrating the high-density SNP datafiles with call rates > 60% could be uploaded into GEDmatch to search and identify reliable matches with direct-to-consumer datafiles based on shared centimorgan regions of similar chromosomes. These results served as the criterion for developing initial acceptance criteria for FGG casework. The fore-mentioned casework criteria were used as guidelines to accept forensic genealogy casework, and the casework evidence samples were processed to develop high-density SNP datafiles to upload into GEDmatch or Family Tree DNA(FTDNA). Using casework blood samples with a minimal acceptance criteria of 0.5 ng DNA, over 90 % of blood casework samples produced valuable SNP datafiles with call rates >60% that could be successfully uploaded to third party databases and produce reliable matches that share at least 20 cM between the experimental datafile and direct to consumer datafiles. Similar pass rates are observed using DNA from spermatocytes and bone evidence samples. Pass rates were slightly higher from microarray case work then genomic sequencing analysis. Analysis of 170 casework samples demonstrate that DNA samples with 1.0 ng DNA and degradation indices >10 did generate valuable SNP datafiles with call rates >60% and capable of identifying valuable matches in the third-party databases. The observation that useful SNP datafiles can be generated from low quantity or low-quality DNA indicate the importance of establishing casework acceptance criteria and genome testing guidelines.

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### **Forensic Genealogy, SNPs, Case Acceptance Criteria**



## **B147 Internal Validation of the ForenSeq™ Kintelligence Kit for Application to Forensic Genetic Genealogy Cases**

*Michelle Peck, MFS\*, Signature Science, Charlottesville, VA; Stephen Turner, PhD, Signature Science, Charlottesville, VA; V. Peter Nagraj, MS, Signature Science, Charlottesville, VA; David Russell, MS, Signature Science, Charlottesville, VA; Jessica Bouchet, BS, Signature Science, Charlottesville, VA, Amy Holmes, PhD, Signature Science, Charlottesville, VA; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX; Jennifer Snedeker, BS, Sam Houston State University, Huntsville, TX; Carmen Reedy, PhD, Signature Science, Charlottesville, VA; Christina Neal, MS, Signature Science, Charlottesville, VA*

**Learning Overview:** After attending this presentation, attendees will understand the performance of the ForenSeq Kintelligence Kit in an internal validation, its compatibility with challenging forensic samples, and how it can be applied to forensic genetic genealogy (FGG) cases.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how a laboratory can validate the ForenSeq Kintelligence Kit and implement it for forensic genetic genealogy cases.

The ForenSeq Kintelligence Kit (Verogen), a targeted amplification approach for SNP genotyping, was specifically designed for forensic genetic genealogy cases.<sup>1,2</sup> The design took into consideration compatibility with the most challenging forensic samples (i.e., low input and degraded samples) and does not include medically relevant SNPs due to genetic privacy considerations. The 10,230 SNP markers in the panel can be used for distant kinship matching, phenotype, and ancestry inference.

Initial optimization testing identified modifications to improve performance and efficiency, particularly with low input samples. First, a decreased final library elution volume increased library yields and subsequently total SNP calls, while maintaining sufficient library volume. Secondly, a modified pooling and dilution strategy was applied to maximize molarity input and better normalize pooled samples. Finally, a range of sample pool multiplexing levels was evaluated to best use the flow cell capacity, while balancing cost and time considerations.

Internal validation testing in accordance with SWGDAM standards demonstrated sensitivity down to 0.05 ng of DNA, high precision and accuracy, the ability to detect mixtures, negligible contamination, and high performance with nonprobative samples.

Within sensitivity testing, calls rates of over 95% and 97% were observed with inputs of 0.05 ng and  $\geq 0.1$  ng (up to 1.0 ng) with control DNA, respectively. Concordance rates were  $92.8 \pm 0.8\%$  for 0.05 ng,  $97.0 \pm 0.3\%$  for 0.1 ng, and  $99.0 \pm 0.15\%$  for 0.25 ng and 1.0 ng. The majority of discordance observed was due to allelic drop-out attributable to low DNA input. To help overcome these stochastic effects, combining replicate FASTQs was evaluated to determine the impact on both call rates and concordance. Further, multiplexing levels of both 2-plex and 4-plex were assessed to maximize performance and efficiency.

Nonprobative testing evaluated the ForenSeq Kintelligence Kit with extracts from bones, shell casings, and tape collections. Bones were tested at range of input from 0.1 to 1.0 ng, including samples extracted with two different methods and replicates at different inputs. Six bone samples (i.e., burned, cremated, surface decomposition, and embalmed), starting as low as 0.042 ng/ $\mu$ L, were evaluated at 1.0 ng DNA input. These samples resulted in  $9,934 \pm 258$  called SNP sites, equivalent to a  $97.1 \pm 2.5\%$  call rate, when sequenced in a 4-sample multiplex. Further, a tape collection extract at 1.0 ng input yielded 9,873 total SNPs, including 77 of 78 ancestry and phenotype markers.

The ForenSeq Kintelligence Kit demonstrated robust performance in this internal validation testing. As the laboratory seeks to apply this method, implementation considerations include compatible sample types, likelihood of informative database results, cost, and time. One of the major advantages of the workflow, particularly for challenging samples, is the large sample volume it can accommodate; up to 25  $\mu$ L of a sample. The multiplexing level will be assessed for each case to determine the optimal processing strategy to maximize data recovery while balancing cost and time considerations. The ForenSeq Kintelligence Kit will serve as a viable option for FGG cases, particularly for cases with low input.

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## **SNP Genotyping, Massively Parallel Sequencing, Forensic Genetic Genealogy**

**B148 An Analysis of the Genealogy Process in Investigative Genetic Genealogy**

*Su Erturk, BS, Stanford University, Stanford, CA; Lawrence Wein, PhD\*, Stanford University, Stanford, CA*

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**Learning Overview:** The goal of this presentation is to provide attendees with an understanding of the genealogy process in investigative genetic genealogy (IGG), to learn how this process can be mathematically modeled and optimized, and to gain new insights into how the genealogy process can become more effective (solve more cases) and efficient (take less time).

**Impact Statement:** This presentation will impact the forensic science community by: 1) showing for the first time how the genealogy process within IGG can be mathematically modeled and optimized, 2) estimating key model parameters (e.g., the probability of identifying a match, someone's parents or someone's children) from data on 17 cases, 3) computing the probability of solving a case as a function of the amount of work invested, and 4) introducing a new strategy that attempts to optimize the tradeoff between solving more cases and doing less work.

The genealogy process is typically the most time-consuming part of (and a limiting factor in the success of) IGG. The objective of this study is to develop a systematic approach to efficiently perform the genealogy portion of investigative genetic genealogy.

The genealogy process is formulated as a two-stage mathematical model: an ascending stage that attempts to find the most recent common ancestors (MRCAs) between the unknown individual and each investigated match, and a descending stage that searches for a marriage among the descendants of the MRCAs. For any given set of investigated matches (and their genetic distance to the unknown individual), the probability of identifying the unknown individual and the expected amount of work (i.e., size of the final family tree) are computed. We consider three strategies, each operating under three informational scenarios. Two of the three strategies descend only from known MRCAs, and differ by how they seek MRCAs: one seeks MRCAs directly between the unknown individual and the match, while the other seeks MRCAs between two different matches.

The third strategy is a greedy policy that myopically chooses the next action (i.e., which match to investigate, which MRCA to descend from, or whether to terminate the investigation) by maximizing the marginal benefit-to-cost ratio (i.e., the increase in probability of finding the unknown individual divided by the expected additional work required) given the current system state. The three informational scenarios relate to what is known about an identified match: unobserved cluster (e.g., cannot determine which groups of matches descend from the same set of great-grandparents) and relationship to the unknown individual, observed cluster and unobserved relationship, and observed cluster and relationship.

Data from 17 unidentified remains cases (eight solved, nine unsolved) from DNA Doe Project are used to estimate the model's parameters and compare the three strategies under the three informational scenarios.

Our study highlights the benefits achieved by the proposed greedy strategy; of aggressively descending from a match's ancestor that is not known for certain to be a MRCA with the unknown individual. It also assesses the value of the AutoCluster and AutoKinship tools used in GEDmatch, and reveals how our proposed greedy strategy exploits these tools.

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**Crime Solving, Forensic Genealogy, Probabilistic Analysis**

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## **B149 A Theft of Consent: How FGG solved one the most difficult unsolved serial rapist cases in Texas and Louisiana**

*Leighton D'Antoni, JD\*, Dallas County District Attorney's Office, Dallas, TX*

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**Learning Overview:** Attendees can expect to learn about Forensic Genetic Genealogy (FGG) and advances in DNA as applied to criminal investigations of unsolved cold cases.

**Impact Statement:** This presentation will educate attendees and highlight the fact that FGG is the future to solving the most difficult cold cases that have been previously unsolvable.

David Hawkins was a serial rapist who committed over 30 violent home invasion rapes all across north Texas and north Louisiana from 1980-1985. It wasn't until the FBI teamed up with the Dallas County DA's Office to use FGG to identify Hawkins, arrest him, persuade him to confess to over 30 cases, and ultimately accept 4 Life Sentences for his crimes.

"David Hawkins is a monster and deserves every single second of those four life sentence he was sentenced to today," said prosecutor Leighton D'Antoni.

In court, one survivor from a 1985 aggravated sexual assault asked the judge to let Hawkins remove his face mask.

"I have never seen his face. He had on a ski mask that night," the woman told the judge. The judge told Hawkins he could remove his mask. "I have been searching for you for 35 years," the woman said. "I always wanted a face and a name for the sicko who raped me." "I would not let it define me," she said of the assault. "The search for you has empowered me."

**December 18, 1982:** A woman woke up to Hawkins pulling the covers off her and pointing a gun to her face. Hawkins raped the woman and then stole money and jewelry. At one point, the woman's roommate came home.

Hawkins held the roommate at gunpoint and told her to undress. He then raped the other woman again. He left after telling both women to be quiet.

**January 3, 1983:** At 3:00 a woman was asleep in her bed when she was awakened by Hawkins lying on top of her saying "If you scream, I'll kill you." Hawkins was wearing a ski mask, gloves and had a gun. Hawkins tied the woman to her bed, covered her head, and sexually assaulted her by penetrating her vagina with his penis. The suspect then demanded the complainant's money and jewelry but did not take anything. Hawkins untied the woman and forced her to douche. Hawkins told her to not to call the police "if she knew what was good for her."

**August 18, 1983:** A woman was asleep in her bed when she heard her door open. A masked man came in and forced her to lay on her stomach. The man tied her hands behind her back. He raped her, while threatening to shoot her if she didn't cooperate, according to a police report.

Afterward, the man untied the woman and told her to wash up. He then took some jewelry and said, "Don't tell anyone this happened. Just forget about it."

**April 27, 1985:** A woman woke up to Hawkins wearing a turquoise ski mask with pink around the eyes and dark blue gloves. Holding a gun, he told her, "Either get [expletive] or get killed."

Hawkins put the woman on her stomach and tied her hands behind her back. He then threatened to gag her if she didn't stop crying and stay quiet."

The woman called 911. She underwent a sexual assault exam at the hospital. The rape kit, the woman's bedsheets and another washcloth she used were collected and tested for DNA. A profile was identified, but not matched to a suspect for over 30 years.

**Went cold again:** The 4 DNA profile from the 4 Dallas rapes matched 2 unsolved cases in Shreveport, LA. Though the six rapes were linked through DNA testing in 2005, the cases went cold again.

The investigation was re-opened in 2020. The FBI teamed up with Dallas County DA SAKI team to use FGG to identify a family tree, which led investigators to Hawkins. It was the first time in Dallas history, FGG had been used to identify a suspect.

D'Antoni, the lead prosecutor, said "It was truly the only way these cases were going to be solved." "FGG is the future of solving previously unsolvable cold cases. I am excited that here in Dallas County, we will be national leaders using this tool to solve violent cold cases."

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**FGG , Serial, DNA**

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**B150 Nuclear DNA Single Nucleotide Polymorphism (SNP) Profiles Derived From a Human Hair Shaft**

*Daniele Podini, PhD\*, George Washington University, Washington, DC; Elaine Lewis, PhD, George Washington University, Washington, DC*

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**Learning Overview:** Attendees of this presentation will learn of a new approach to obtain nuclear DNA SNP profiles from hair shaft using the power of massively parallel sequencing (MPS).

**Impact Statement:** The ability to obtain a nuclear DNA SNP profile from hair found at crime scenes or from unidentified individuals would improve our ability to identify the contributor through DNA analyses.

Shed hair is a frequent item found at crime scenes, and yet has not been reliably useful in DNA analyses. The shed hair is usually lacking the root, containing the nuclei. The remaining hair shaft contains both mitochondrial and nuclear DNA, but in low amounts. In addition, the DNA is frequently degraded, leaving fragments of approximately 100 bases, and in otherwise poor quality for analyses. The small size of the DNA fragments precludes analysis of standard short tandem repeats.

This study describes our steps to overcome the difficulties inherent in obtaining a nuclear DNA profile from hair shaft. DNA is extracted from 5-10 cm of hair shaft using the method of Brandhagen et al.<sup>1</sup> The DNA recovered is quantified for both mitochondrial and nuclear DNA using a qPCR assay derived from an assay described by the C. Calloway laboratory.<sup>2</sup> Recovery of DNA is highly variable among hairs, ranging from 0-10 pg nuclear DNA per cm of hair. The DNA hair samples have been used successfully to detect and identify nuclear SNPs using Snapshot assays developed in our laboratory, demonstrating the presence of nuclear DNA.

DNA samples from the same donor are combined to reach several hundred picograms and are used as templates for DNA library construction. In library construction, adapters compatible with the Ion Torrent S5 MPS system are ligated onto the ends of the hair DNA, followed by 18-20 rounds of PCR amplification. The libraries are analyzed by a bioanalyzer and have an average fragment size of ~140-160 bases.

The libraries are subsequently used in capture hybridization enrichment assays. The target sequences are a panel of 238 SNPs and microhaplotypes selected for high discrimination power. The regions surrounding and including the variants were used as templates for synthesis of RNA “baits” which specifically hybridize to the corresponding DNA segments of the hair-derived libraries. Following hybridization reactions, the hybridized hair-derived DNA is recovered, amplified, and sequenced with the Ion Torrent S5 MPS system. Analysis of the sequences reveals the genotype of the donor DNA for the variants included in the original panel.

The further development of the techniques described in this study should provide a new strategy for human identification from hair.

**Reference(s):**

1. Brandhagen, M.D.; Loreille, O.; Irwin, J.A. (2018) Fragmented nuclear DNA is the predominant genetic material in human hair shafts. *Genes* 9:640 doi:10.3390/genes9120640.
2. Wisner, M.; Erlich, H.; Shih, S.; Calloway, C. (2021) Resolution of mitochondrial DNA mixtures using a probe capture next generation sequencing system and phylogenetic-based software. *Forensic Science International: Genetics* 53: <https://doi.org/10.1016/j.fsigen.2021.102531>.

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**Hair Shaft, SNPs, Microhaplotypes**

## **B151 A Comprehensive Evaluation of the ForenSeq™ DNA Signature Prep Kit on the MiSeq® FGx™ Next Generation Sequencing (NGS) System**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the performance of the ForenSeq DNA Signature Prep Kit on the MiSeq Next-Generation Sequencing (NGS) platform.

**Impact Statement:** This presentation will impact the forensic science community by presenting an evaluation of the ForenSeq DNA Signature Prep Kit Primer Set B and comparison to gold standard capillary electrophoresis (CE) instrument. The information from this presentation can be used by others to help determine possible important points of information, such as limit of detection, for the MiSeq FGx NGS system.

CE is the gold standard and is a widely used platform in forensic DNA laboratories for human identification purposes. While CE enables fragment analysis, NGS technology, also known as massively parallel sequencing (MPS), has grown in popularity among crime and research DNA laboratories. This is due to its enhanced capability to multiplex and run in parallel hundreds of forensically relevant markers including identifying short tandem repeats (STRs) and phenotypic and ancestral marker single nucleotide polymorphisms (SNPs). In addition, it allows information to be obtained on individual sequences of STR markers. MiSeq FGx (Illumina) system is currently one of the most used platforms in forensic DNA practice along with the ForenSeq DNA Signature Prep Kit (Verogen). This kit contains Primer set B, which offers more forensic insight into phenotypic and ancestry information of individuals than Primer set A which includes only STR and SNP markers. In this study, the ForenSeq DNA Signature Prep Kit Primer set B will be evaluated on the MiSeq system and compared to the performance of the CE system.<sup>1-4</sup>

In this study, male and female reference samples were collected from buccal swabs, extracted using the EZ1 Advanced XL (Qiagen), and quantified on 7500 Real-Time PCR (Applied Biosystems™) system using the Quantifiler Trio DNA Quantification Kit. All single-source and mixed samples are to be amplified with the GlobalFiler™ PCR Amplification Kit and run on the 3500 Series Genetic Analyzer (ABI) for DNA typing. The same samples will then be run on the MiSeq FGx Sequencing System, the results will be compared, and stutter analysis was performed. The evaluation of the ForenSeq DNA Signature Prep Kit primer set B included concordance, sensitivity, reproducibility, the limit of detection, and mixtures studies. The sensitivity of this NGS kit will be tested at 1,000 pg, 500 pg, 250 pg, 125 pg, 63 pg, and 31 pg. An assay of mixtures representative of commonly encountered mixed samples at 1 ng of total input DNA will be prepared. These include two-person, three-person, and four-person mixtures. A total of 18 mixtures are to be prepared for this research. For two-person mixtures, male:male and male:female mixtures at 1:1, 1:5, 1:10, 1:20, 1:50 and 1:100 ratios will be prepared. Three-person mixtures will be simulated as female:male:male at four different ratios of 1:1:1, 5:1:1, 10:5:1, and 20:5:1. Four-person mixtures will be prepared as female:female:male:male mixtures at ratios of 1:1:1:1, 5:5:1:1, and 10:5:1:1.

The reference samples will be the first run on the CE and then on the NGS using the standard flow cell kit. Quantification and CE typing results of the single-source DNA samples are to no contamination issues and that mixture ratios are correct. Preliminary sequencing results on these samples showed a higher level of sensitivity and detection limit of the MiSeq system than that of the CE platform. Moreover, an initial set of mixture samples at 1:1, 1:5, 1:50, 1:1:1, and 10:1:1 ratios were prepared and first run on the CE to help determine the actual ratios of the mixtures before the NGS run. CE mixture results confirmed the expected ratios of all mixed samples, which were then run on the MiSeq. Overall, the preliminary NGS data showed a higher sensitivity to that of the CE.

In conclusion, these preliminary results highlighted an enhanced capability of the ForenSeq DNA Signature Prep Kit primer set B to detect and deconvolute mixture contributors on the NGS platform in comparison to CE fragment analysis. A comprehensive mixture and stutter analysis are ongoing to fully evaluate this sequencing kit.

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### **ForenSeq™ DNA Signature Prep Kit, Next Generation Sequencing (NGS), Capillary Electrophoresis**

## **B152 Developmental Validation of the Illumina® Infinium™ Assay Using the Global Screening Array (GSA) on the iScan® System for Use in Forensic Laboratories**

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**Learning Overview:** This presentation will provide an overview of the developmental validation of the Infinium Global Screening Array-24 v3.0 Kit [GSA] on the iScan array system; a microarray-based genome-wide SNP genotyping workflow. It will provide valuable insight into what forensic practitioners can expect from microarray-based genotyping data.

**Impact Statement:** This presentation will provide valuable insight into what forensic practitioners can expect from microarray-based genotyping data.

This developmental validation study aimed to provide the forensic community with a validated microarray-based genome-wide SNP genotyping workflow for DNA inputs consistent with forensic sample types. Most labs performing microarray-based SNP genotyping are clinical and lack a forensic framework. Our approach was to evaluate the Infinium Global Screening Array-24 v3.0 Kit [GSA, Illumina] on the iScan array system [Illumina], focusing on shifting applicability from clinical laboratories to the forensic community. To date, there is no existing guidance on how to validate microarray-based genome-wide SNP genotyping within the forensic community. This validation was performed guided by standards (FBI QAS, SWGDAM) accepted by the forensic community with the intended application to challenging cases crime labs encounter. This will allow greater confidence in the investigative leads developed by law enforcement agencies, as the data used to develop such leads will have been generated under the same scientific standards already established within other human identification methods.

The following studies were performed as part of the developmental validation: sensitivity, precision, and accuracy (repeatability and reproducibility), mixtures, degradation, species specificity, contamination, case-type samples, and array stability-post processing. Throughout all studies, except for the case-type study, extensively characterized human genomes for which high-confidence variant calls are known were used. Concordance was assessed using the NIST/Genome-in-a-bottle (GIAB) sample call sets as truth data for each sample.

For the sensitivity study DNA inputs from 200 to 0.2 ng were assessed using NA12878 [Coriell]. This evaluation demonstrated call rates of >99% and >95% for inputs  $\geq 1$  and 0.2 ng, respectively. Additionally, results were highly concordant at DNA inputs as low as 0.2 ng: <0.001% discordance for all replicates down to 1 ng, and <0.5% discordance at 0.2 ng. For the precision and accuracy (repeatability and reproducibility) studies, when comparing sample genotypes to the NIST/GIAB sequencing data, average concordance rates were 99.2% across all samples. Comparing duplicate samples to each other showed a concordance rate of >99.8% across all samples. The repeatability and reproducibility studies demonstrated reliable and consistent call rates and high concordance, regardless of operator.

In the mixture study, the profile generated from samples of a 3:1 mixture of NA24631 to NA12878 (major to minor contributor) was on average 98.85% concordant with gold standard data for NA24631; the 9:1 mixture ratio was on average 99.99% concordant. This demonstrated that at a mixture ratio of 3:1 or greater between the major and minor contributors, the resulting major contributor's profile is accurate. The data generated during the degradation study was unexpected; samples known to have been severely degraded produced genotyping data with call rates similar to pristine samples. However, examination of concordance data showed that the genotype calls were less accurate as the samples became more degraded. Lower heterozygosity was observed in the degraded samples (average of 7%) compared to the GIAB population set (average of 17.3%). This suggests that allelic dropout is responsible for the noticeable shift to false homozygous calls, accounting for the high call rate yet discordant SNP data.

This validation characterized the performance of forensic samples on the GSA platform, providing valuable insight into what forensic practitioners can expect from microarray-based genotyping data. Thresholds for call rate, heterozygosity, and overall signal (fluorescence) were established to assess the data and determine suitability of samples for the workflow.

### **Developmental Validation, Forensic Genetic Genealogy, SNP Genotyping**

## B153 Joint Data Analysis of DNA Mixtures Using Probabilistic Genotyping

*Sara Antillon, MS, George Mason University, Fairfax, VA; Jennifer Hornyak Bracamontes, MS, Cybergenetics, Pittsburgh, PA; Kevin Miller, PhD, Hamilton Robotics, Reno, NV; Mark Perlin PhD, MD, PhD\*, Cybergenetics, Pittsburgh, PA; Mark Wilson, PhD, George Mason University, Fairfax, VA*

**Learning Overview:** After attending this presentation, attendees will understand how probabilistic genotyping (PG) methods can conduct joint data analysis of multiple DNA data samples to produce more informative genotypes and likelihood ratio (LR) match statistics.

**Impact Statement:** This presentation will impact the forensic science community by showing how joint data analysis can obtain more information by analyzing multiple DNA data samples at the same time, particularly helpful for interpreting mixed or low template DNA (LT-DNA) samples.

Polymerase chain reaction (PCR) is a random DNA amplification process that inherently produces unequal amounts of amplified allele amplicons. Scientists can extract all the DNA identification information from complex mixtures of several people, or from just a few cells in the sample, only by probability modeling of the short tandem repeat (STR) PCR experiment. This forensic innovation is called “probabilistic” genotyping, since it has a computer assign accurate probabilities to all genotype possibilities, instead of discarding DNA evidence as inconclusive, or reporting inaccurate LR values.

When analyzing one DNA sample, PG computer analysis usually yields more information than human review of the same STR data. However, a joint likelihood function can simultaneously examine multiple data items. This joint analysis can extract even more information from DNA evidence data. Information was quantified here by the magnitude of  $\log(\text{LR})$  match statistic, measured in ban units.

The study examined DNA mixture samples containing two, three, four, and five contributors, at both high and low amounts of DNA. The selected samples were interpreted using the TrueAllele® PG method, comparing single and joint analysis. There were three sample groupings: complementary mixture weight (MW), template concentration, and complex mixtures. The combined joint analysis showed an overall increase in genotype and match information, relative to single analysis of individual evidence samples.

For samples with complementary MWs, on average joint analysis gave 10 ban more information than single analysis, when a contributor was uninformative or a minor component. For example, single analysis of a 50% two-person mixture contributor produced a  $\log(\text{LR})$  of 11 ban, and analysis of a four-person 5% contributor mixture gave a  $\log(\text{LR})$  of 3 ban. Joint analysis produced a  $\log(\text{LR})$  of 18 ban for this shared contributor, which is a 7 ban and 15 ban respective increase in identification information over the single analysis for the 50% and 5% fractions.

Single sample analysis of LT-DNA gave minor contributors having  $\log(\text{LR})$  values as low as 3 ban. Jointly analyzing three LT-DNA data experiments for a minor 10% component produced a  $\log(\text{LR})$  around 14 ban, increasing match information 11 ban relative to single analysis.

In a terrorist case, a matchstick was recovered from the scene of a torched getaway car. Human review of the low copy number (LCN) amplifications was uninformative. TrueAllele computer review of each individual LCN amplification gave LR values around ten. However, joint TrueAllele data analysis of the three LCN amplifications produced a higher LR value of a million, which brought the DNA evidence back into the case.

Joint data analysis generally increases genotype and DNA match information. Two common scenarios are when (a) contributor mixture weight is different across the samples, and (b) a small amount of the same contributor is present in multiple samples. In these scenarios, joint data analysis may be the only way to obtain informative LR results for a suspect from the DNA evidence.

Collecting more LT-DNA samples up front can lead to more informative joint analysis downstream. Separate STR analysis of the samples permits joint computer analysis, whereas a pooled sample PCR does not. Planning for joint data analysis can constructively change initial sample collection and laboratory procedures.

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### Probabilistic Genotyping, Joint Data Analysis, Forensic DNA

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**B154 An Evaluation of the QIAamplifier® 96 Thermal Cycler**

*Madeline Roman, PhD\**, Signature Science, LLC, Austin, TX; *Josh Abernathy, MSFS*, QIAGEN, Germantown, MD; *Bryan Davis, BS*, QIAGEN, Germantown, MD

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how different thermal cyclers perform with forensic STR chemistries and what studies are necessary to ensure that new thermal cyclers are performing comparably to existing ones.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new thermal cycler for STR amplification in crime laboratories.

A typical forensic DNA workflow consists of DNA extraction, quantification, amplification of STRs, and capillary electrophoresis. Multiple STR amplification chemistries are commercially available, and the quality and accuracy of STR results is affected by the thermal cycler used for amplification. The purpose of this study was to test the compatibility of a newly available thermal cycler, the QIAamplifier® 96 (QIAGEN), with STR multiplexes used for human identification. Sensitivity, accuracy, reproducibility, and inhibition studies were performed using the Investigator® 24plex QS kit (QIAGEN), and results from the QIAamplifier® 96 were compared to results from the Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific). In addition, compatibility with direct PCR was tested using the Investigator® 24plex GO! Kit.

The sensitivity study consisted of two male reference samples amplified in triplicate at each input ranging from 63 pg to 1 ng. For both thermal cyclers, dropout occurred at 63 and 125 pg for one sample, and full profiles were recovered for the other sample throughout the entire range of DNA inputs. No differences were observed in profile completeness, average peak heights, average peak height ratios, or intra-color balance between the QIAamplifier® and Veriti thermal cyclers using the F statistic (95% confidence interval). The reproducibility study involved amplification of twelve replicates of positive control DNA on each thermal cycler at 0.5 ng. All replicates resulted in 100% profile completeness, and there was no significant difference between average peak heights, average peak height ratios, and intra-color balance between thermal cyclers (F-test). Inhibition was tested by addition of three concentrations of hematin to positive control samples prior to amplification. All samples resulted in 100% allele recovery, with no significant differences between average peak heights, average peak height ratios, and intra-color balance between thermal cyclers (F-test). Finally, direct amplification was assessed using three buccal swabs amplified in triplicate with the Investigator® 24plex GO! kit. All samples and replicates resulted in 100% allele recovery using both thermal cyclers. There was no significant difference between average peak heights or average peak height ratios, and all genotypes were concordant with the reference profiles (F-test). Throughout all studies, all negative controls resulted in no allele calls, and no new reproducible STR artifacts were observed.

The concordance of results between the QIAamplifier® 96 and Veriti 96-Well Thermal Cycler indicates that the QIAamplifier® 96 is compatible with STR amplification chemistries and may be used as an alternative to other thermal cyclers in forensic casework laboratories. Though only two QIAGEN STR chemistries were analyzed during this study, it is likely that other STR chemistries would perform similarly well and generate comparable results using the QIAamplifier® compared to other thermal cyclers commonly utilized by forensic laboratories.

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**STR, PCR, Thermal Cycler**



## B155 Toward a *Sample In-Answer Out* Centrifugal Microfluidic Platform for Nucleic Acid Detection

Leah Dignan, BSc\*, University of Virginia, Charlottesville, VA; Michael Woolf, PhD, University of Virginia, Charlottesville, VA; Will Treene, University of Virginia, Charlottesville, VA; James Landers, PhD, University of Virginia, Charlottesville, VA

**Learning Overview:** After attending this presentation, attendees will have been introduced to a novel, rotationally-driven *sample in-answer out* microdevice that integrates and expedites nucleic acid extraction, amplification, and detection.

**Impact Statement:** This presentation will impact the forensic science community by introducing a microfluidic platform for comprehensive integration of nucleic acid preparation and detection that is amenable for use outside of traditional laboratory settings.

Nucleic acid amplification tests (NAATs) are ubiquitous, yet complex, multi-step processes encompassing liberation (e.g., cell disruption), extraction, quantification, amplification, and ultimately detection of nucleic acids (NAs). Despite an increasing demand for portable NAATs, the majority of existing techniques remain tethered to centralized laboratories, where their time-consuming and labor-intensive workflows must be conducted by trained analysts.<sup>1</sup> Microfluidic systems offer rapid, cost-effective, and portable alternatives to laboratory-based analyses that are readily amenable to use by non-technical personnel. Ideally, fully automated *sample in-answer out* platforms would permit comprehensive NA processing, from sample preparation through interpretation of results, with no user intervention.<sup>1</sup> Here, we describe our work towards one such portable, automatable centrifugal microfluidic platform to integrate and expedite detection of human DNA.

Although there has been much focus on microfluidic adaptations of NA amplification and detection, the advancement of NA extraction has lagged behind.<sup>2</sup> Thus, initial work was centered on developing and characterizing on-disc enzymatic direct-from-swab DNA extraction. The resulting genetic material was compatible with 'gold standard' real-time and multiplexed polymerase chain reaction (PCR) techniques. In addition, extracted DNA was amenable to loop-mediated isothermal amplification (LAMP), which is especially well-positioned for point-of-need use due to simplified hardware requirements (e.g., no thermal cycling) and compatibility with visual, colorimetric detection.<sup>3</sup> Following initial in-tube pilot studies showing successful colorimetric LAMP, the associated chemistry was adapted to a centrifugal microfluidic format. Specifically, by targeting and amplifying the thyroid peroxidase (TPOX) locus, the presence of human DNA was visually detected.

The ability to perform all NA processing operations on a single centrifugal microdevice is enabled by laser-actuated valve opening and channel closures, as well as a novel method for fluid displacement towards the center of rotation using reagents stored on-board.<sup>4</sup> The latter technique is beneficial for such multi-step assays because it acts counter to centrifugal force, allowing for continued on-disc processing without integration of bulky external hardware (e.g., pneumatic pumps). Previous work demonstrated proof-of-concept but relied on highly corrosive reagents and complex fabrication methods.<sup>5</sup> Here, an alternative strategy leveraging simple, on-board storage of safe-to-handle reagents that exhibits compatibility with amplification-based NA detection is demonstrated. Subsequent on-board coupling of NA preparation, inward fluid displacement, and colorimetric LAMP represents significant progress towards a fully automated *sample in-answer out* system for fieldable genetic analysis.

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### Nucleic Acid Amplification Tests (NAATs), Microfluidics, Forensic DNA

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## **B156 Using a Non-Destructive Method to Estimate the Number of Contributors and Predict DNA Yield in Touch DNA Mixtures**

*Susan Greenspoon, PhD, Virginia Department of Forensic Science, Richmond, VA; Christopher Ehrhardt, PhD, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA; Christin Lee Master\*, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA; Sarah Ingram, PhD, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA*

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**Learning Overview:** After this presentation, attendees will become more familiar with the application of antibody probes for DNA casework. Specifically, antibody probes can hybridize known target proteins in a cell sample without destroying cell structure. Applying antibodies to target proteins/hormones in human epithelial cells, the individualized signaling peaks can represent each contributor, and the shift/intensity of fluorescent can predict the amount of DNA to each person in a mixture sample.

**Impact Statement:** This project investigates a new pre-DNA-profiling method that can screen for touch DNA in epithelial cell mixtures rapidly and in a non-destructive manner. This new approach applies fluorescent-tagged intra-cellular molecules to estimate both contributor number and DNA content, preserving the DNA from touch evidence, while still providing information about the samples before STR analysis. This technique has the potential to increase the information available for mixture touch evidence prior to DNA profiling, which may assist with sample triaging as well as data analysis and interpretation.

DNA deposited by handling an item is also referred to as touch DNA. It can play an important role in forensic science since it is an extremely common form of evidence left at crime scenes. However, most touch DNA obtained from an object is low template and has been contacted by multiple individuals. Touch DNA samples are very frequently difficult to interpret and distinguish contributors, and these challenges can decrease the success rate of casework. Currently, the most common method to estimate the number of contributors and the amount of DNA for each contributor from touch DNA mixture samples is based on DNA quantitation and short tandem repeat (STR) analysis; however, there are some limitations, making a definitive interpretation difficult. Therefore, applying a non-destructive pre-DNA analysis technique that can differentiate cell populations and predict DNA yield may improve the casework process and increase success in interpretation.

Recent published research demonstrated that antibody probes can differentiate cell populations in mixture samples, however, little has been published applying this technique to epithelial touch cell mixtures. Cholesterol-based hormones such as testosterone and dihydrotestosterone (DHT), and proteins including cytokeratins (CK) inside epidermal cells have been demonstrated to be a personalized signature in human populations. Both hormone and cytoskeletal targets are stable for long periods of time, making them feasible candidates to act as a marker to differentiate cell populations. The binding efficacy of testosterone and DHT for each individual in an epithelial mixture sample and the correlation between predicting DNA yield versus actual DNA yield still remains unknown. Furthermore, there are a small number of reports on CK in the forensic field. Thus, much remains to be discovered regarding its potential applications.

In this study, swabs of epithelial cells collected directly from each volunteer's hands were cut in half, with one-half stained with fluorescently tagged anti-testosterone and anti-DHT antibodies or stained with anti-cytokeratin antibody. The other half of the swabs were extracted for DNA. Fluorescence histograms were generated for the stained sample of each epithelial cell population using flow cytometry. Staining patterns in the fluorescent histograms were observed that differed among the contributors in mixture samples. This result demonstrated that cholesterol-based hormones were able to differentiate cell populations and thereby distinguish each contributor. Furthermore, in the DNA quantitation experiment, the remaining half of the epithelial cell swabs were extracted for DNA and quantitated using a qPCR assay. The quantitation data showed that the DNA amount correlated with the shift in fluorescence in the histograms; those samples with a higher concentration of DNA showed a larger shift in the fluorescent signal (greater fluorescence). This finding suggested that using antibody probes can be used to predict DNA yield before any DNA has been extracted. Moreover, the cell staining and flow cytometry is a non-destructive method of cell analysis. Thus, these findings indicated that antibody hybridization has the potential to serve as a non-destructive technique prior to STR analysis to enhance the probative value of biological evidence.

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### **Touch DNA, Testosterone and Dihydrotestosterone, Cytokeratins**

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## **B157 An Evaluation of Microhaplotype Markers in Kinship Analysis in a Korean Population**

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**Learning Overview:** After attending this presentation, the attendees will understand the potential of microhaplotypes for use in kinship analysis, and their power of resolution in analysis for distinguishing true relatives from the unrelated compared to when using the current genetic markers, STRs and SNPs.

**Impact Statement:** This presentation will impact the forensic science community by serving the statistical values of relatedness evaluated based on likelihood ratio (LR) for various familial relationships from actual Korean pedigrees, including parent-child, full-siblings, uncle-nephew, grandparent-grandchild, and first cousins, which can be considered for kinship determination.

Microhaplotypes are a novel-type of genetic markers emerging in forensic genetics recently, which may supplement existing markers for different forensic purposes. A microhaplotype, also known as a microhap, is defined by two or more closely linked SNPs associated in multiple allelic combinations. Microhaplotypes have advantages of both STRs and SNPs, including lower mutation rates, absence of stutter peaks, small amplicon size less than 300 bp usually, and multiple alleles. Those features can provide usefulness for human identification, relationship identification, and mixture deconvolution.

Here, we present the distributions of likelihood ratio for different familial relationships by analyzing 51 microhap markers using massively parallel sequencing technology. The markers analyzed were selected from the literature, data were analyzed using STRait Razor 3.0, and each kinship was assessed against unrelated pairs based on likelihood ratio using Familias 3 software. For comparison of the usefulness of microhaps in kinship analysis, tested pairs were also analyzed using two different genetic marker systems consisting of 22 STRs and ~160 SNPs from commercial kits, respectively. In the results, all pairs in kinship of parent-child and full-siblings were clearly resolved from the unrelated with a high sensitivity. For distant relationships, uncle-nephew or grandparent-grandchild, the number of pairs within the 'grey zone' where uncertainty exists due to overlapping of the LR distribution curves of true relatives and the unrelated were decreased compared to when using STRs. Compared to when using SNPs, the resolution somewhat was similar, which may be due to the total number of SNP similar in analysis in each system. A higher resolution for distant relationships can be expected with more microhap markers on a single panel with the current set of markers, and it is believed that these microhaplotype markers have a potential for use in kinship analysis of various familial relationships for forensic application.

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### **Microhaplotypes, Kinship Analysis, Korean**

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### **B158 Examining the Ancestry of the Spring Street Presbyterian Church Burial Vault Population Using Mitochondrial DNA (mtDNA) Analysis**

*Stephanie Gladysck, PhD\**, Madonna University, Livonia, MI; *Shannon A. Novak, PhD*, Syracuse University, Syracuse, NY; *Jodi Lynn Barta, PhD\**, Madonna University, Livonia, MI

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**Learning Overview:** Attendees will learn about DNA extraction, amplification, and analysis that effectively allows for ancestry identification of aged, degraded bone samples.

**Impact Statement:** This presentation will outline methods that have been implemented to extract genomic DNA from challenging, low copy number bone samples in order to amplify, analyze, and determine maternal ancestry of individuals based on mitochondrial DNA. Aged and degraded bone samples are some of the most difficult to analyze and sharing successful methods within the forensic science community helps lead to improvements in forensic science practice.

During the winter of 2006, where SoHo Towers in New York City now stand on the corner of Spring and Varick Streets in Manhattan, construction crews uncovered four underground burial vaults. The vaults belonged to the historic Spring Street Presbyterian Church and subsequent excavation yielded the commingled remains of over 200 individuals ranging from infants to the elderly. The Spring Street Presbyterian Church (SSPC) was a prominent abolitionist institution from the early to mid-19th century. Church pastors were known for admitting to the congregation African Americans, the working poor, and immigrants despite accusations and backlash from members of the community, including riots that resulted in the burning of the church and one pastor's home. Few archival records remain, though New York City burial records suggest that two "Blacks" were interred in the church vaults. Burial practices in New York City at the time were more commonly segregated, and it remains unclear if there was differential access to burial in the SSPC vaults or if segregation occurred within them. Therefore, we ask: did the church practice what they preached in their burial practices? The goal of this project is to answer this question through the analysis of DNA for ancestry determination. Despite the age of the bones and the likelihood that the DNA was subject to degradation during freeze-thaw cycles in the burial environment, methods traditionally used for ancient DNA analysis were performed on the remains of these individuals in an attempt to identify the geographical origin of their maternal ancestors. This research successfully utilized whole genomic extraction, polymerase chain reaction amplification, sequencing, and analysis of mitochondrial DNA from the skeletal remains of 13 individuals to determine the ancestry of members of this population. Results of these analyses reveal that a diverse group of individuals found their final resting place in the SSPC burial vaults.

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#### **MtDNA, Degraded DNA, Ancestry Determination**

## **B159 Contemporary Population Genetics Data and Haplogroup Prediction Using 27 Y-Chromosomal Short Tandem Repeat (Y-STR) Loci in the Croatian Population**

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**Learning Overview:** This presentation will offer attendees a better understanding of how analysis of increased number of STR loci can expand informativeness of Y chromosome haplogroups, offering more accurate results as well as higher probability of detecting all (including rare) Y haplotypes.

**Impact Statement:** This presentation will provide an update of forensic and population genetic parameters regarding Y chromosome markers which is important for forensic scientists.

Almost 50% of the Y chromosome consists of repetitive sequences.<sup>1</sup> When it comes to more mutable repetitive elements, prominent examples are short tandem repeats (STRs), with an average mutation frequency of ~0.2% per generation.<sup>2</sup> These markers are representing important genetic tools for various studies; therefore, Y chromosome has an important role in forensic analyses, in cases of paternity testing, in the process of human identification, but also in human migration studies.<sup>3</sup> Therefore, updating of all forensic and population genetic parameters regarding Y chromosome markers is mandatory for forensic scientists. Y chromosome haplogroups can be successfully predicted from Y-STR haplotypes using different software packages.

Buccal swab samples were collected from 518 unrelated male individuals from five Croatian cities and their close surroundings: Hvar (island Hvar, Split-Dalmatia County) n=104, Split (Split-Dalmatia County) n=105, Varaždin (Varaždin County) n=100, Osijek (Osijek-Baranja County) n=110, Pula (Istria County) n=99. Y-STR genotyping was performed using Yfiler™ Plus PCR Amplification Kit (Applied Biosystems) targeting 27 Y-STR loci.<sup>4</sup> The number of alleles and different haplotypes, allele and haplotype frequencies, and gene and haplotype diversity were estimated in order to assess the intrapopulation diversity. The evolutionary history was inferred for both set of markers using the neighbor-joining (NJ) method of phylogenetic tree construction in MEGAX, whereby the optimal tree is shown.<sup>5,6</sup> Genetic distances between groups of males and between populations were quantified by  $R_{st}$  using AMOVA online tool from the Y Chromosome Haplotype Reference Database – YHRD.<sup>7</sup> In addition, associated probability values (P values) with 10,000 permutations were included for the studied populations. Genetic distances were used to generate MDS plots for the comparison of population haplotype data from YHRD. AMOVA analysis was done with two population groups. The first comparison for analysis was made using the original number of the Yfiler Plus kit. The second comparison was made with a reduced number of markers using the Yfiler marker set. Y-chromosomal haplogroup prediction using allele frequencies on 518 Yfiler Plus profiles was performed using Whit Athey's Haplogroup Predictor v5, an algorithm based on Bayesian-allele-frequency approach.<sup>8</sup> The Y chromosomal STR data of the present study are submitted to Y-STR Haplotype Reference Database (YHRD).

A total of 513 different haplotypes were detected in the study, with 508 unique haplotypes and 5 haplotypes appearing twice. In addition, the largest number of alleles was recorded on DYS481 with 14 detected alleles. Three loci had the smallest number of alleles, namely DYS393, DYS437 and Y-GATA-H4 with 4 alleles each. Average genetic diversity for the study population was 0.656 across all loci, ranging from 0.251 at the locus DYS392 to 0.886 at DYS481. Eleven haplotypes with possible new (not confirmed within YHRD) microvariants are detected, requiring additional sequencing.

Both population comparisons point towards a general conclusion that geographically closer populations show higher degree of genetic relatedness. Population comparisons using comparative analysis methods presented above show very low genetic differentiation between the currently analyzed population in this part of the Europe. The results revealed the existence of 15 different haplogroups, with I2a, R1a and E1b1b being the most prevalent with frequencies of 39.00%, 24.32% and 10.81% respectively. Compared to the previously published studies on Croatian population based on Y-SNP and Y-STR data, this study represents an upgrade of molecular genetic data with increased number of loci, thus offering more accurate results as well as higher probability of detecting all (including rare) Y chromosome haplogroups.

**Reference(s):**

1. Jobling, M.A.; Tyler-Smith, C. (2003) The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet* 4: 598-612.
  2. Ballantyne, K.N.; Goedbloed, M.; Fang, R.; Schaap, O.; Lao, O., et al (2010) Mutability of Y-chromosomal microsatellites: Rates, characteristics, molecular bases, and forensic implications. *Am J Hum Genet* 87: 341-53.
  3. Marjanović, D.; Primorac, D.; Dogan, S. (2018). *Forensic Genetics: Theory and Application*. Sarajevo, Bosnia and Herzegovina: International Burch University, ISBN: 978-9958-834-63-9.
  4. *Yfiler™ Plus PCR Amplification Kit User Guide, Revision D*, 2019. Life Technologies Ltd [https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2F4485610\\_YfilerPlus\\_UG.pdf](https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2F4485610_YfilerPlus_UG.pdf).
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  6. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* 35: 1547-49.
  7. Willuweit, S.; Roewer, L. International Forensic YCUG (2007) Y chromosome haplotype reference database (YHRD): update. *Forensic Sci Int Genet* 1: 83-7.
  8. Athey, T.W. (2013). *Whit Athey's Haplogroup Predictor*. Accessed, August 24, 2021.
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**Croatia, Y-Chromosome, Diversity**

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**B160 Rapidly Mutating Y-Chromosomal Short Tandem Repeats (RM Y-STRs) in Punjabi Deep-Rooted Endogamous Pedigrees From Pakistan**

*Shahid Nazir, PhD\*, University of Health Sciences, Lahore, Punjab, Pakistan*

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**Learning Overview: Attendees will learn:** (1) in sexual assault cases it is very tricky to separate male relatives of the same family but using RMSTRs we can differentiate between them, (2) the diffraction power of RM Y-STR loci In Pakistani populations, (3) population genetic analysis of Pakistani population using RM Y-STRs allele frequency data, and (4) this data will be very valuable to law enforcement agencies and criminal justice system.

**Impact Statement:** The overall objective of this exploration is to validate a single plex assay, which can validate 13 RM Y-STR loci in the Pakistani population till fourth generation. Such assay will aid a breakthrough in human population's studies as well as forensic applications.

The objective of the study was to explore the discrimination power of RM Y-STRs in Pakistani population till four-generation. This study can be utilized effectively in the following ways: (1) In sexual assault incidents, it is very tricky to separate male relatives of the same family but using RMSTRs we can differentiate between them, (2) to investigate the RM Y-STR loci in Pakistani populations, (3) leading a population genetic study of Pakistani inhabitants using RM Y-STRs allele frequency data, (4) assist the Pakistan justice system and law enforcement agencies.

Short tandem repeat polymorphisms on the male specific portion of the human Y-chromosome (Y-STRs) are important in many areas of human genetics. Y chromosomal STRs being normally utilized in the field of forensic exhibit low haplotype diversity in endogamous populations and fail to discriminate among male relatives from same pedigree. Rapidly mutating Y-STRs (RM Y-STRs) have been paid much attention in last decade. These 13 RM Y STRs have high mutation rates ( $>10^{-2}$ ) and have considerably higher haplotype diversity and discrimination capacity than conventionally used Y-STRs showing remake-able power, when it comes to differentiation in paternal lineages in endogamous populations. Separation of male relatives is usually impossible with standard Y-STRs. Previously, we have analyzed 2–4 generation, 99 pedigrees covering 1568 pairs of men covering 1–6 meioses from all over Pakistan and 216 male relatives from 18 deep rooted endogamous Sindhi pedigrees covering 1-7 meioses.

Here we are presenting 861 pairs of men from 63 endogamous pedigrees covering 1-6 meioses from Punjabi population of Punjab, Pakistan. Mutations were frequently observed at DYF399 and DYF403 while no mutation was observed at DYS526a/b. The rate of differentiation ranged from 29.70% (first meiosis) to 80.95% (fifth meiosis) while overall (1 to 6 meiosis) differentiation was 59.46%. Combining previously published data with newly generated data, an overall differentiation rate was 38.79% based on 5176 pairs of men related by 1–20 meioses, while Y-filer differentiation was 9.24% based on 3864 pairs. Using father-son pair data from the present and previous studies, we also provide updated RM Y-STR mutation rates.

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**Rapidly Mutating, Short Tandem Repeats, Y-Chromosome**

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**B161 Developing Effective and Sustainable Teaching Labs for Forensic DNA Courses**

*Catherine Cupples Connon, PhD\*, Virginia Commonwealth University, Richmond, VA*

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**Learning Overview:** After attending this presentation, attendees will be aware of some of the challenges involved with (and solutions to) developing forensic DNA laboratories for teaching purposes. These laboratories should be robust enough for students with little to no laboratory experience, offer an introductory level of analysis, and be sustainable long-term from a cost perspective.

**Impact Statement:** This presentation will impact the forensic science educational community by offering a low-cost assay for human-specific DNA quantitation, as well as a cost saving approach to STR profiling via robust quarter PCR reactions for blood and buccal samples.

Developing forensic science laboratories for educational purposes can be challenging for a variety of reasons. Robust protocols that work well for the average, relatively inexperienced student are a necessity, and fortunately, there are a number of quality options to choose from. However, many of these come with a hefty price tag, especially for forensic DNA analysis. Sustaining these costs long-term presents additional challenges. One remedy is to only process a small number of samples to keep costs low, but this is often not the best option as students will have a more well-rounded experience if they can process more than one or two samples. Other options include utilizing robust homebrew assays and/or consuming less reagent per sample. This talk will address some specific solutions to these issues, including the adoption of low-cost, human-specific qPCR assays and quarter reactions for STR profiling. The methods employed here also take into account the inexperience of most students, both from a laboratory technique perspective, as well as from a data analysis perspective.

Studies were conducted to assess the effectiveness of a low-cost human-specific qPCR assay utilizing Alu repeats and SYBR Green detection.<sup>1</sup> Two PCR master mixes (Power SYBR<sup>®</sup> Green and PowerUp<sup>™</sup> SYBR<sup>®</sup> Green) were evaluated using full (25 $\mu$ L) and half (12 $\mu$ L) reactions in combination with eight versus five DNA standards processed in singlicate and duplicate. For each combination of variables, three standard curves were generated and used to quantify five DNA extracts (loaded in duplicate) of varying quantities. Evaluations included assessing the standard curves ( $R^2$ , slope, y-intercept, and degree of manipulation needed to pass) and reproducibility of quantitation. PowerUp<sup>™</sup> SYBR<sup>®</sup> Green outperformed Power SYBR<sup>®</sup> Green on all combinations of standards for full reactions. Half reactions were not viable under any circumstance. From the successful qPCR setups, amplification of 0.7-1.1ng of DNA yielded high quality full profiles using the AmpF/STR<sup>®</sup> SGM Plus<sup>™</sup> PCR Amplification Kit.

The other area of our teaching laboratory that was altered was amplification of STR profiles. Low volume quarter reactions (6.2 $\mu$ L) based on an earlier study were applied to the AmpF/STR<sup>®</sup> COfiler<sup>®</sup> and SGM Plus<sup>™</sup> PCR Amplification Kits for buccal swabs and blood on FTA cards.<sup>2</sup> Resulting STR profiles were analyzed from 143 student-generated amplifications. These were assessed for overall interpretability (interpretable, uninterpretable, or no results) and basic metrics. Of all student-generated profiles, 76% were interpretable and, of those, 71% had full profiles. Interpretable profiles exhibited average peak heights of 1872 rfu, average PHR of 87.7%, and an average of seven artifacts per sample, the vast majority of which were pull-up.

These studies demonstrate that a low-cost qPCR assay and a lower cost quarter reaction for STR amplification are viable options for students in a forensic biology teaching laboratory. These assays are robust enough that even students with little to no laboratory experience can be successful and have data to evaluate. Furthermore, though these are not the top-of-the-line options for analysis of casework, they provide students with a nice introduction to forensic DNA analysis that is not as overwhelming as qPCR assays with numerous detectors and/or STR profiles with >20 loci.

**Reference(s):**

1. Nicklas, J.A.; Buel, E. Development of an Alu-based, real-time PCR method for quantitation of human DNA in forensic samples. *J Forensic Sci.* 2003 Sep;48(5):936-44.
2. Connon, C.C.; LeFebvre, A.K.; Benjamin, R.C. Validation of Low Volume, Fast PCR Amplification of STR Loci for DNA Reference Samples. *Journal of Forensic, Legal & Investigative Sciences* 2016; 2:008.

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**Education, QPCR, DNA**



## B162 The Evaluation of Cannabinoid Synthase Polymorphisms for Distinguishing Between Marijuana and Hemp

Ya-Chih Cheng, MS\*, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX; Sarah Kerrigan PhD, Sam Houston State University, Huntsville, TX; Madeline Roman, PhD, Signature Science, Austin, TX

**Learning Overview:** After attending this presentation, attendees will understand the limitation of a previous method utilizing tetrahydrocannabinolic acid synthase (THCAS) polymorphisms to differentiate marijuana and hemp and the potential of high-throughput analysis of three synthase genes to reveal novel polymorphisms to identify cannabis crop type or strain.

**Impact Statement:** Cannabis *sativa*, which can be cultivated as hemp or marijuana, has been one of the most controversial plants in the United States. The recent passage of the Agriculture Improvement Act of 2018 that legalized hemp charges law enforcement with differentiating the two crop types. This presentation will impact the forensic science community by demonstrating the applicability of the previously reported single nucleotide polymorphism (SNP) marker system and the importance of expanding sample varieties and performing more robust sequencing to evaluate genetic markers for the differentiation of hemp and marijuana.

Genetic markers are desirable for determining crop type in sample types inconducive to chemical analysis, such as trace residues, small leaf fragments, immature crops, aged samples, seeds, and root material. Cannabinoid synthase genes are considered the key enzymes that determine a particular cultivar's chemical composition or chemotype. The use of cannabinoid synthase genes to predict *C. sativa* chemotype has been proposed in several studies. However, existing methods for crop type differentiation based on the past theory could lack specificity and accuracy, and a broader range of cannabis varieties must be considered when examining cannabis-based genetic markers.

This study optimized and evaluated a previously reported SNP assay for determining *C. sativa* crop type (marijuana or hemp).<sup>1</sup> In order to achieve the most accurate results, the assay was first optimized to ensure sufficient amplification. The method was then applied to reference *C. sativa* samples from NIDA (National Institute on Drug Abuse) and NIST (National Institute of Standards and Technology), hemp samples from the USA and Canada, and marijuana samples from USA-Mexico and Chile. The SNP assay correctly identified crop type in most seized samples and commercial hemp based on the original model with active versus inactive isoforms of THCAS. However, nine out of eleven marijuana reference samples appeared to be classified as hemp. In addition, several hemp seeds, which generally have low levels of cannabinoids, were classified as marijuana, and two strains of legal CBG hemp flowers were classified as marijuana. The results demonstrated the inaccuracy of the model assuming active- and inactive-THCAS. This suggested that the reported THCAS SNP markers might not represent the genetic variation of the synthase gene (including gene expression at the time of harvest) and are not directly related to the cannabinoid content in the cannabis plant. This study also performed qualitative chemical analysis on hemp and reference samples to better understand the relationship between the chemotype and genotype.

In order to develop more robust genetic markers, sequencing of the three synthase genes of the cannabis reference materials was performed. This data serves as a preliminary design for a future next generation sequencing custom panel targeting cannabinoid synthase genes. This custom panel will serve as a comprehensive analysis of the three synthase genes in order to discover novel polymorphisms between crop types and/or strains.

Overall, this presentation highlights the limitation of a previously published method for the differentiation of marijuana and hemp and the potential to find novel polymorphisms between crop types and strains with a high throughput sequencing technique.

### Reference(s):

- <sup>1</sup> D. Rotherham; S.A. Harbison. Differentiation of drug and non-drug Cannabis using a single nucleotide polymorphism. *Forensic Sci. Int.*, vol. 207, pp. 193–197, 2011, doi: 10.1016/j.forsciint.2010.10.006.

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### *Cannabis Sativa*, Cannabinoid Synthase Gene, Single Nucleotide Polymorphisms

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**B163 An Evaluation of Chloroplast DNA Barcoding Markers to Individualize *Papaver Somniferum* for Forensic Intelligence Purposes**

*Kari Graham, BS\**, Sam Houston State University, Huntsville, TX; *Rachel Houston, PhD*, Sam Houston State University, Huntsville, TX

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**Learning Overview:** After attending this presentation, attendees will understand a novel research project which seeks to: (1) provide a comprehensive screen of chloroplast DNA barcoding markers for the individualization of *Papaver somniferum* samples, and (2) show how these regions can be used for source attribution and case linkage of seized opium poppy and heroin samples.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview of regions within the chloroplast genome of *Papaver Somniferum* that can be used for investigative leads for law enforcement to help link cases, distributors, growers, or individual samples

*Papaver somniferum*, commonly known as opium poppy, is the source of natural opiates like morphine and other alkaloids that produce analgesic and psychotropic effects. These compounds can be chemically separated and modified to produce opioids of varying strength and effectiveness as pain relievers in modern medicine. Unfortunately, the widespread use of these medications has resulted in an epidemic in the United States that causes over 40,000 deaths a year.

The Drug Enforcement Agency (DEA) has recently developed the Heroin Source Program (HSP). One goal of this program is to chemically analyze and identify the impurities of heroin to track and source seized heroin samples. While this program is moderately successful, using genetic information from *P. somniferum* could strengthen the program. Until recently, it was hypothesized that usable DNA could not be obtained from heroin due to the high temperatures and pH ranges used in heroin production. However, a study by Marciano et al. demonstrated that *P. somniferum* DNA suitable for genetic analysis could be extracted from heroin samples.<sup>1</sup> This demonstrated the possibility of utilizing DNA to individualize heroin samples to determine origin. In addition, chloroplast DNA (cpDNA) markers may help to provide the investigative leads needed for source attribution or case linkage.

This project aims to evaluate various chloroplast markers for intraspecific variation from various sources of *P. somniferum*. The chosen barcoding regions were previously shown to distinguish between the closely related *P. somniferum* and *P. setigerum*.<sup>2</sup> *P. somniferum* seeds were obtained from a variety of sources and extracted. Primers were designed to target various barcoding regions of the *P. somniferum* chloroplast genome: *psbA-trnH*, *trnL-trnF*, *trnE-trnT*, *rpl32-trnL*, *ndhF-rpl32*, *rps16-trnQ*, and *petA-psbJ*. These barcoding regions were then sequenced using Sanger sequencing and screened against published cpDNA sequences for polymorphisms between the varieties of *P. somniferum* samples. Of the barcoding regions screened, two markers, *trnH-psbA* and *petA-psbJ*, showed promise in differentiating samples of *P. somniferum*. This presentation will discuss the process of screening the barcoding regions and designing and optimizing custom Sanger sequencing to identify the cpDNA barcoding markers most helpful in distinguishing between *P. somniferum* samples of different origins. This is an ongoing project, and future research will focus on investigating other promising cpDNA barcoding markers, developing and validating a comprehensive analytical tool used for biogeographical determination and case linkage where opium poppy and its various derivatives are seized.

**Reference(s):**

- <sup>1</sup> Marciano, M.A.; Paniker, S.X.; Liddil, G.D.; Lindgren, D.; Sweder, K.S. Development of a Method to Extract Opium Poppy (*Papaver somniferum* L.) DNA from Heroin. *Scientific Reports*. 2018; 8(2590).
- <sup>2</sup> Liu, L.; Du, Y.; Shen, C.; Li, R.; Lee, J.; Li, P. The complete chloroplast genome of *Papaver setigerum* and comparative analyses in *Papaveraceae*. *Genetics and Molecular Biology*. 2020; 43(3).

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**Chloroplast DNA Barcoding, Heroin, *Papaver Somniferum***

## B164 Development and Validation of Rapid Color Test for Acetic Anhydride

Muhammad Chaudhary, MPhil\*, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Tahir Jamshaid, MPhil, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Rabia Naveed, MPhil, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Muhamamd Irfan Ashiq, PhD, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Mohammad Tahir, PhD, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan

**Learning Overview:** After attending this presentation, attendees will be able to know the method validation protocol and economical identification of acetic anhydride samples using simple color test.

**Impact Statement:** Forensic science community will be able to overcome the challenging identification of acetic anhydride by using this color test in laboratories. Forensic science community will prefer to use this simple and cost-effective test with suitable degree of reliability rather than use of Hi-Tech expensive methods.

Acetic anhydride (AA) (CH<sub>3</sub>CO)<sub>2</sub>O, is a colorless liquid with pungent vinegar like odor. It is the largest commercially produced carboxylic acid anhydride.<sup>1</sup> Its use in illegal synthesis of heroin from morphine is important in illicit drug market. The United Nation (UN) has implemented the control of AA as essential precursor around the world.<sup>2</sup> About 100-250 liters of AA can be used in conversion of opium to about 100 kilograms of heroin.<sup>3</sup> Pakistan is also a key market for AA trafficking and its use in heroin production. Various seizures have been carried out in Pakistan during last years which were identified by using UNODC precursor identification kit.

AA can be identified in forensic samples using various chemical spot test and quantified. In 1925, Whitford et al reported a method for rapid and accurate determination of acetic anhydride using anhydrous oxalic acid in the presence of Pyridine as catalyst.<sup>4</sup> Other method include titration for determination of AA in samples. Rosenbaum's macro procedure was adapted in first method involving 10-25 mg sample with known amount of oxalic acid and titrating with permanganate. The other method involved reaction of 10-15 mg sample with pyridine-oxalic acid mixture in vessel and ultimately determining carbon dioxide gravimetrically using sodium hydroxide-coated asbestos.<sup>5</sup> All such method are laborious and demand the use of various chemicals in controlled manner. AA is identified using Drug and Precursor Identification Kits developed by UNODC which is expensive.

The method is based on reaction of anhydrous Citric acid (few milligrams) with suspected acetic anhydride sample (more than 20 uL) in the presence of pyridine solvent (two drops) producing yellow to orange red color which on heating turns purple/violet using water bath at 80-90°C. It is pertinent to mention that acetic acid (major degradation product) did not react to produce any color change. Furthermore, various liquids and solvents (similar to acetic anhydride) were tested for selectivity and did not produce any color change. When common drugs of abuse were tested in the presence of acetic anhydride, none of those interfered with the reaction. LOD of this method was found to be 20 uL which is equivalent to 21.6 mg of AA. Furthermore, method was found reproducible when used by different analysts in the laboratory. This method is suitable for screening of acetic anhydride in suspected samples.

Based on the validation study, it is concluded that this test is selective, robust and fit for use in the casework for presumptive screening of acetic anhydride.

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### Presumptive Test, Acetic Anhydride, Citric Acid Test

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**B165 A Journey: The International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17020: 2012 Accreditation for Crime Scene Units in Latin America**

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**Learning Overview:** After attending this poster presentation, attendees will have a better understanding of the importance of pursuing accreditation for Crime Scene Investigation Units applying ILAC G19:2014, ISO/IEC 17020:2012 and accreditation requirements from an accrediting body.

**Impact Statement:** This poster presentation will impact the forensic science community by presenting the journey of Crime Scene Investigation Units toward accreditation and the correction actions of non-conformities detected during the process.

Over decades, there have been many discussions in different countries in Latin America regarding standards for crime scene investigations. The main reason for requiring such standards is primarily due to the critics and review of this activity and the results within respective judicial systems and stakeholders. In these countries, none of national accreditation bodies have been intervening on this issue under the International Laboratory Accreditation Cooperation (ILAC) Guide 19: Module for Forensic Science Process, and the progress had been relatively slow. In USA, accreditation bodies have been in the accreditation process for Crime Scene Units (CSU) under ISO/IEC 17020 with the first units accredited in 2012; Puerto Rico in February 2012 and Texas in March 2012. Outside the USA, Mexico obtained their first accreditation of CSI in September 2015 and Panama in July 2021.

To determine the best way forward for Central and South America, and to ensure that a common approach would be adopted, International Narcotics and Law Enforcement Affairs (INL) and the International Criminal Investigative Training Assistance Program (ICITAP) from the US Department of Justice agreed in a joint venture to prepare Crime Scene Units for accreditation throughout Central and South America, to ensure common interpretation of the international standards and to obtain valid, reproducible and quality of results. The outcome was an understanding between INL and ICITAP USDOJ, recommending the international quality standard for inspecting bodies, ISO/IEC 17020:2012, as the most appropriate standard for crime scene investigation. At the same time, a working group composed of forensic advisors from USA and Mexico, with experience in Quality Systems, Crime Scene Investigations (CSI) and other Forensic Disciplines, aided in the interpretation of ISO/IEC17020 for processing crime scenes. These forensic advisors help in the development of framework of quality standards for forensic science to meet the needs of the Criminal Justice System and is designed to give the public confidence that forensic science used in the investigation and prosecution process is fit for purpose.

The Forensic Advisors contracted by federal contractors are working with INL and ICITAP to support the development of quality standards framework inclusive of the quality management system: development and review of technical procedures, instructions, guides, manuals, forms; GAP analysis, training, consultation, advice towards validation, verification, equipment (instruments, reagents, certified materials, consumables, etc.) corrective actions, and continued education toward accreditation. This framework from INL and ICITAP USDOJ, together with the contracted Forensic Science Advisors sets out the quality requirements for providers of forensic science services into the criminal justice system. It is a firm understanding that all forensic science providers shall gain accreditation to ISO/IEC 17020:2012 and/or ISO/IEC 17025:2017, as appropriate, for their scope of work, and that individual practitioners are able to demonstrate a level of competence commensurate with the requisite of the forensic discipline and country legislation. The scope of this poster presentation is focused on crime scene investigation, restricted to ISO/IEC 17020:2012 and how to obtain the accreditation of this field.

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**Crime Scene Investigation (CSI), Accreditation, ISO/IEC ISO 17020:2012**

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## B166 The Effect of Household Cleaning Agents on the Fluorescein Presumptive Blood Test

*River Williams\*, University of Findlay, Findlay, OH; Jaymelee Kim, PhD, University of Findlay, Findlay, OH*

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**Learning Overview:** After attending this presentation, attendees will have an increased understanding of the impact that cleaning agents can have on blood detection using fluorescein.

**Impact on the Forensic Science Community:** This study contributes findings regarding the impacts of common cleaning agents on the accuracy of presumptive blood detection.

Blood evidence found at crime scenes can often provide valuable information to investigators. To detect the presence of blood, presumptive blood tests are performed at the scene. One common test uses a fluorescein reagent. When in contact with blood, oxidation of the hemoglobin causes the sample to fluoresce under ultraviolet light.<sup>1</sup> Because household cleaning agents may be used to obscure evidence of a crime, investigators need to ensure that the fluorescent reaction in this test is not impeded with cleaning agents.

Building on studies conducted by Adams, Rancourt, and Christensen and Signaevsky, this project uses ten common household cleaners selected to undergo the fluorescein test with synthetic human blood present.<sup>2,3</sup> After a preliminary trial, all samples had a positive reaction with the fluorescein, including the negative controls containing only cleaning agents. This indicates that these cleaning agents have components with inherent fluorescent capabilities, which could disrupt the determination of the presence of blood at crime scenes. In a second phase of the experiment, the same cleaning agents were prepared at 5%, 25%, and 50% dilution with deionized water. These dilutions were used as samples for carrying out the fluorescein procedure without blood being present and evaluated for the presence of absence of fluorescence under UV light. In this trial, the samples all exhibited fluorescence including negative controls. This is consistent with the original findings by Signaevsky, which showed abnormalities in some negative controls; however, the findings are inconsistent with Adams, Rancourt, and Christensen, which did not have any abnormal negative controls.<sup>2,3</sup>

Future applications of this study would be to test more cleaning agents to determine if they have fluorescent capabilities.

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### Blood Detection, Fluorescein, Cleaning Agents

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## **B167 Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS): Optimized Dispersion and Double-Pass Raman Analysis**

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**Learning Overview:** After attending this presentation, attendees will gain knowledge of the optimized parameters for the PCRS analysis of soil minerals to achieve maximized discrimination, through particle dispersion and Raman microspectroscopy.

**Impact Statement:** This presentation will impact the forensic science community by detailing the optimized methods for soil mineral particle dispersal and PCRS analysis, which are necessary for maximizing the discriminating power of PCRS for forensic soil analysis.

The analysis of soils has become a neglected field in forensic science due to the perception of current practices as being overly time-consuming, subjective, and ineffective. The potential value of soil evidence is incontrovertible, and thus several analytical approaches are being researched which can revitalize the use of soil as forensic evidence. Particle Correlated Raman Spectroscopy (PCRS) is a recently developed technique that has demonstrated value for forensic soil analysis, as it combines particle size distribution and morphological measurements with chemical identification by Raman spectroscopy. This technique is non-destructive, rapid, and can be automated; thus, PCRS may provide more detailed information than traditional methods about a soil sample.

Previous research identified optimized parameters for the identification of soil minerals via PCRS. Such parameters included the laser wavelength and power, magnification, exposure time, and grating. These parameters, which were initially optimized using 10 diverse minerals, have been applied to the 60 most commonly encountered minerals. Traditional figures of merit and response surface modeling of a multi-level experimental design were used to confirm the optimized Raman collection parameters. It was concluded that a double-pass Raman analysis, using two laser wavelengths, is needed to identify the majority of the soil minerals. Further, total Raman acquisition times of 1 second per particle were achieved which would enable a large number of mineral grains to be identified in a reasonable time period, thus making it possible to have robust datasets for subsequent statistical analysis.

Prior research also began the process of parameter optimization for the vacuum dispersion of soil samples. Proper dispersion must be attained to minimize overlapping or clustered particles so that data can be accurately obtained for individual particles. Soil samples were prepared for analysis by washing alone, washing and sieving, or analyzed with no further preparation. Each sample was then dispersed onto glass analytical plates with various combinations of vacuum dispersion parameters (sample volume, vacuum pressure, and time for dispersal and settling). The dispersion was assessed microscopically via PCRS for reproducibility, uniformity, dispersion density, and the maintenance of particle morphological characteristics throughout the processing. PCRS was also used to analyze four morphological characteristics of these dispersed samples – area, diameter, ellipse ratio, and circularity – at three points on the analytical plate – center, 2 cm from center, and 3.5 cm from center.

The results from this research, optimized PCRS analysis parameters, will next be applied to a large collection of soil samples to create a robust database combined with statistical analysis to support the evidentiary significance of soil. Optimization of sample preparation, dispersal, and Raman analysis parameters are necessary to take full advantage of the discriminating power of PCRS for soil analysis.

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### **Forensic Soil Analysis, Vacuum Dispersion, Raman Spectroscopy**

## B168 Feature Occurrence and Error Rates in Textile Physical Fit Comparisons

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**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the error rates in physical fit comparisons of textiles and the occurrence rate of specific features that influence the decision-making process.

**Impact Statement:** This presentation will impact the forensic science community by responding to calls for research into the development of standardized methods and identification of error rates in physical fit comparisons.

Textiles are a frequently encountered form of evidence at crime scenes. Textiles can take the form of clothing, bedding, carpeting, and more. This type of evidence is particularly useful in physical fit comparisons. A physical fit directly links two items of evidence as originating from the same common source and is considered as the highest degree of association between two items due to the belief that it is extremely unlikely that two unrelated fractured items fit together as one. Despite the potential value of this evidence, the validity and reliability of physical fit comparisons has not been widely studied, and there are no consensus-based standard protocols or criteria for conducting these types of comparisons.

Due to the common nature of textiles at crime scenes, and the potential value in identifying a physical fit between two separated items, a study was designed to evaluate performance rates in textile physical fit comparisons. A systematic method was adapted for use with textiles where the analyst divides the length of the textile edge into ten comparison areas.<sup>1</sup> The analyst then examines the quality of the physical fit between the two textiles in question at each comparison area and classifies each individual area as a “fit” or a “non-fit” using a score of 1 (fit), 0 (non-fit), or 0.5 (inconclusive), respectively, along with documenting the observed features. The numbers assigned to each area are then summed and divided by 10 (the total number of comparison areas) to reach an overall edge similarity score. Variability in the construction and composition of textiles also required the identification of factors that affect the suitability of specific types of textiles to undergo a physical fit comparison. Specific features that either enhance or diminish the quality of a physical fit between textiles are also documented in a systematic method.

A textile comparison set of 200 samples was created using five clothing articles of 100% cotton knit clothing. Three fabrics were solid colored, while two items exhibited a multi-colored pattern in their construction. The sample set was evenly split between hand-torn and stabbed separation methods to represent two common ways that textiles can be separated in a crime. The ground truth of the comparisons was kept blind to the analyst, who then conducted all 200 comparisons using the systematic method described previously. The analyst documented specific features throughout the comparison process that either increased or decreased their confidence in either a fit or non-fit decision.

The analyst completed the stabbed textile subset with 100% accuracy, and therefore no misclassifications. The hand-torn textile subset was completed with 96.84% accuracy. The analyst reported that 2 comparisons were inconclusive, while one true positive comparison was reported as a non-fit, resulting in a false negative. Moreover, five comparisons in the hand-torn subset, not included in the above performance rates, were identified as not suitable for comparison due to extreme distortion from the tearing process masking potential features that could have informed the analyst’s decision-making process. These performance rates are comparable to a previous study in our group using the same method on a subset of 100 sample of varying compositions, which all exhibited inter- and intra-examiner accuracies between 87.20% and 98.00%.

Several prominent textile features were identified throughout the comparison process, including weave direction alignment, pattern/print alignment, fiber gap alignment, curling, secondary tearing, fluorescence, and extreme distortion, that were all influential in the decision-making process. Weave direction proved to be an especially informative feature, as it affects the alignment or misalignment of the direction of the fiber construction at the comparison edge.

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### Physical Fits, Textiles, Error Rate

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## B169 Detection of Animal Blood Using Green Synthesized SERS

*Geraldine Monjardez; PhD\*, Sam Houston State University, Huntsville, TX; Erin Bruner, MSc, Texas DPS Houston Crime Lab, Houston, TX; Rajesh Balaraman, PhD, Vironova, Cambridge, MA; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** After attending the presentation, attendees will gain a better understanding of the practical use of green synthesized SERS to collect and identify body fluids from crime scenes.

**Impact Statement:** This presentation will impact the forensic science community by presenting an efficient and rapid method to detect non-human blood.

While DNA profiling is commonly used to identify individuals, identifying the type of body fluid recovered from crime scenes before it can be used for DNA typing may be critical to help qualifying its relevancy to the case. While typical approaches that rely on biochemical tests to detect and identify body fluids already exist, they present several drawbacks. Surface-enhanced Raman spectroscopy (SERS) has been used to detect trace level of chemical and biological analytes using their characteristic vibrational signatures. This study aims to develop a method of collection and identification for non-human blood using green synthesized SERS swabs.

Following a general movement in the natural sciences to move toward more sustainable practices, silver nanoparticles were successfully synthesized directly on nylon evidence swabs using a biosynthesis reaction and curcumin as the reducing agent to form the nanoparticles. The nanoparticles were characterized on the swabs by Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDS). The SEM images confirmed the presence of the nanoparticles adhering to the fibers of the swabs and their rough surface texture. The nanoparticles were observed to be dispersed on the fibers, which led to the creation of random hot spots

The detection of bovine blood was investigated by analyzing decreasing volumes of the blood swabbed with the SERS swabs. While swabbing 30  $\mu\text{L}$  and 20  $\mu\text{L}$  of blood resulted in the most resolved spectra, swabbing 10  $\mu\text{L}$  of blood still produced identifiable peaks, with a much lower intensity. Wetted swabs were used to analyze dried bovine bloodstains on two different substrates, 100% cotton fabric and glass microscope slides. The results suggest that the surface on which the dried bloodstain is found may determine whether a wet or a dry SERS swab should be used to collect the sample.

Following the analysis of bovine blood, the SERS swabs were used to collect and analyze horse and sheep blood. The spectra showed that the peak intensity differed between the three species. Dominant bands in some spectra also displayed a shift in dimensions of both left/right. This could be due to several factors, including the complexity and heterogeneity of blood, which could be further increased by the swabs, and the degree of dryness of the sample. Raman bands characteristic of blood were identifiable for each specie, showing that the SERS swabs allowed the successful detection of the three animal blood species.

This study demonstrates the efficiency of the green synthesis to grow the nanoparticles on the swabs and shows their viability to be used as SERS substrates to collect and identify animal blood using Raman spectroscopy.

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**Forensic Science, Animal Blood, SERS**



## B170 The Visualization of Bruises Using an Alternate Light Source (ALS)

Wan Yu, Tan, BSc\*, Boston University, Boston, MA; Karen Kelly, MD, East Carolina University, Greenville, NC; Ann Marie Mires, PhD, Anna Maria College, Paxton, MD; Sabra Jones MSc, Boston University, Boston, MA

**Learning Overview:** Attendees can evaluate the effectiveness of using different ALS wavelengths for the visualization of blunt force injuries (i.e. contusions) to the skin. Since ALS is a standard crime scene equipment, there would be no added cost and this documentation technique could be considered for use in the field prior to transporting the victim to either the hospital or the morgue setting.

**Impact Statement:** With the rise in domestic abuse, victims are usually subjected to physical abuse. This study will enable forensic officers to make use of existing crime scene equipment to enhance the visualization and documentation of bruises on victims of abuse.

With the global pandemic, there has been mandatory movement restrictions by countries around the world. There has also been an increase in domestic abuse; such violence often presents in many forms with physical abuse heading the list.<sup>1-4</sup> This study was conducted to enable forensic officers to make use of existing crime scene equipment to enhance the visualization of bruises on victims of abuse.

When a case of abuse is reported, evidence of the abuse must be documented. Traditional methods of investigation involve questioning the victim or abuser, followed by documentation using photography and note-taking which may not accurately represent the injuries. In addition, the amount of force used, area of injury and the age of the injuries could affect the appearance of blunt force trauma including bruising. At times only redness is observed on the victim's skin making the injury difficult to document; such injuries would constantly be overlooked.<sup>5,6</sup> Alternate Light Source (ALS) is a common, cheap, and effective piece of equipment used by forensic examiners at the crime scene to reveal objects missed by the naked eye. With the use of ALS, the documentation of existing bruises can be enhanced, while bruises that are missed by the naked eye can be revealed.

In this study, the effectiveness of visualization of blunt force injuries (i.e., contusions) to the skin at different ALS wavelengths was evaluated to determine the optimal wavelength for documentation of bruises.<sup>7-10</sup> Bruises were inflicted on 57 participants with no known medical conditions following institutional approval. The participant was in a seated position while a cylindrical ball of ~465 grams was dropped at a height of 1.5 meters through a vertically positioned tube onto the ventral surface of the participant's forearm. The injury site was then observed and documented under white light, 415nm, 460nm and 550nm. Photographs of the forearm were taken under at all wavelengths prior to bruising, immediately after bruising, 3 hours after bruising, and at specific time points over a period of 21 days. The results showed better visualization of the injury observed at a wavelength of 415nm and 460nm.

A blind study was conducted using the same methodology to determine the validity of the experiment. A colleague was briefed and tasked to conduct a blind trial on 12 participants following institutional approval where the researcher has no knowledge on which participant the bruise was inflicted on. Photographic documentation and observations were recorded with the results only made known to the researcher at the end of the experiment. The results showed that the methodology is accurate at about 75%.

This study shows that the use of ALS provided an effective alternative with the visualization and documentation of blunt force traumatic injuries in the form of bruising compared to traditional documentation methods without added cost and should be considered for use in future cases involving trauma and physical abuse. Additionally, since ALS is the standard crime scene equipment, the documentation of bruising by forensic examiners can be initiated in the field prior to transport of victim to either the hospital or morgue setting.

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### Bruise, ALS, Documentation

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## **B171 The Case of a Ferocious Double Homicide: When Your Home Hides the Killer and the Murders' Weapon**

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**Learning Overview:** After attending this presentation, the attendees will be able to better appreciate the methodological approach adopted in a case of double murder performed with multiple different improper weapons.

**Impact Statement:** This presentation will have an impact on the forensic science community by describing an extremely ferocious double murder carried out by associating the use of blunt means with the action of improper piercing objects used to penetrate the victims' neck and abdomen.

Both murders occurred in the same building. The first victim was a 65-year-old woman, mother of the killer, a 34-year-old man with a history of substances abuse. The woman was found near the entrance door of her home at the second floor, prone, in abundant blood, with evident bruising and extensive lacerations at the level of the head and the face, with a broken leg of a chair still inflicted in her abdomen. The walls and the ceiling of the room were extensively smeared with bloodstains; many umbrellas and fragments of wood were found on the floor. The second victim, a 77-year-old woman, lay two floors below. The woman was found on the stairs, half-seated with her neck pierced by two foreign bodies still in site: a wooden one, attributable to a fragment of a chest, and a second metallic one, referable to a candlestick arm. A bloody and deformed umbrella stand, identified as the possible blunt object used to hit the first woman, was found in another apartment, whose door was broken down, together with the remaining parts of the candlestick.

On the basis of the collected testimonies the murderer, once out of the building, stole a car, ran a group of cyclists over, assaulted two elderly people, to finally end his escape by swimming in a lake until being arrested by the police.

The Prosecutor ordered the execution of a judicial autopsy for both corpses, in order to determine the cause of death and obtain further useful information for evidential purposes. The two bodies were PMCT-scanned prior to autopsy and the 3D reconstruction allowed to better define of the foreign bodies' location and the presence of multiple fractures in both cases.

In the first case, external examination allowed to appreciate discontinuity and detachment of a portion of scalp, and the presence of figured lesions depicting the surface of the chair leg on her back. At autopsy, it was possible to clarify the structures damaged by the piercing object, which caused the laceration of the vascular structures of left renal hilum.

Also, in the second case it was possible to identify figured lesions depicting, this time, the tip of the candlestick arm. At autopsy, the dissection of the neck according to Adams technique revealed the laceration of main vascular structures and fractures of mandible and cervical vertebral corpses caused by the metallic means.

The investigations performed, completed with histopathological assessments, allowed to clarify the mechanisms behind the death: in the first case, cause of death was identified in the hemorrhagic cardiovascular collapse due to the laceration of renal hilum, while in the second one asphyxial death because of external compression of airways by hemorrhage and piercing means was supposed.

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### **Blunt Force Injury, Forensic Pathology, Double Homicide**

## B172 Effects of Improper Ammunition Storage

Victoria Andre, MS\*, John Jay College of Criminal Justice, New York, NY; Peter Diaczuk, PhD, John Jay College of Criminal Justice, New York, NY; Patrick McLaughlin, MS, John Jay College of Criminal Justice, New York, NY

**Learning Overview:** After attending this presentation, attendees will become familiar with the effects of improper ammunition storage and the consequences to discharging the compromised ammunition in a firearm.

**Impact Statement:** This presentation will impact the forensic science community by revealing the detrimental nature of outside contaminants permeating cartridges, causing them to malfunction.

Firearms are commonly used by law enforcement, hunters, and civilians either for protection, sport or criminal activity. A firearm is a device used to aim and discharge projectile(s) from a barrel towards a target at high velocity due to gases produced through rapid and confined burning of propellant.<sup>1</sup> The projectiles previously mentioned are one of the four components of ammunition. Ammunition is used in traditional firearms such as rifles and handguns, and consists of a cartridge case, propellant, a bullet (projectile), and primer.<sup>2</sup> The two types of priming systems within the cartridge that are commonly used today are rimfire and centerfire cartridges.

For research purposes, cartridges can be fired to observe and record their performance. To obtain velocity measurements, optical chronographs or a Doppler radar unit such as the LabRadar are commonly used. As with all ammunition, there are malfunctions that can occur. The types of malfunctions that are commonly seen in ammunition are misfire, stovepipe, hang fire, double feed, squib load and misfeed.<sup>3</sup> Improper ammunition storage is also a major factor that plays a role in ammunition failure. Work stress and environmental factors can cause ammunition failure to occur as well.

Another form of improper ammunition storage is where the storage environment being used abruptly changes due to an external liquid or solid material submerging the ammunition. In this scenario, the external liquid or solid material may be able to permeate the ammunition if it is submerged over an extended period of time. As a result, ammunition failure and damage to the firearm may occur if used. While this situation can commonly happen, little research is seen if and how ammunition would function when used in a compromised condition. This study observed how ammunition function is affected when it is improperly stored in the following materials: water, soil, Hoppes 9 Solvent (a gun bore cleaner that contains kerosene, ethyl alcohol, and ammonium hydroxide), and WD-40, (a water-displacing spray that contains aliphatic petroleum distillates and petroleum base oil).

This research showed that although there were distressed cartridges that fired successfully with an expected average velocity measurement obtained, the majority of the cartridges exhibited changes such as ammunition failure, low velocity and anomaly velocity measurements. The types of ammunition failure such as misfires, stovepipe, and double feed were observed with misfires being the most common. All of the distressing materials used had an adverse effect on the cartridges, with Hoppes 9 Solvent being the most detrimental on all cartridge sets. These findings encourage future work to be done to observe any additional detrimental effects of improper ammunition storage in an external liquid or solid.

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### Ammunition Degradation, Environmental Insult, Firearm Malfunction

## B173 The Forensic Utility of the ForenSeq™ MainstAY Kit With Challenging Samples

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**Learning Overview:** After attending this presentation, attendees will better understand the improvements and challenges of implementing Next Generation Sequencing (NGS) in a forensic setting, focusing on the ForenSeq MainstAY kit compared to electrophoresis techniques.

**Impact Statement:** This presentation will impact the forensic science community by presenting data from challenging samples using NGS and comparing this data to results obtained by capillary electrophoresis methods. It will also compare the workflow and discuss the ease of adapting the instrument/method to a crime laboratory workflow.

Most DNA profiles are generated using a standard workflow: extraction, quantitation, amplification, and detection using capillary electrophoresis (CE). For many years, this workflow has provided results for most DNA case working laboratories. However, there continue to be examples where recovered DNA is insufficient to produce complete STR profiles due to low quality, degradation, or mixtures.<sup>1</sup> In these cases, the need to recover more information has grown, and one technique that is more sensitive and yields more genetic data is NGS. Unlike CE platforms, NGS platforms are not limited by dye channel real-estate, and as a result, more amplicons can be simultaneously sequenced and analyzed.<sup>2</sup> In addition, this increased number of amplicons provides analysts more power of discrimination to analyze samples more effectively.

This study focuses on a new NGS assay from Verogen, the ForenSeq MainstAY kit. This kit contains approximately one-fourth the number of markers available in the ForenSeq DNA Signature Prep kit, which has shown to be a powerful tool for recovery in bone samples.<sup>3</sup> The MainstAY kit focuses on identification, including only autosomal STRs and Y-STRs. The decrease in marker complexity increases sensitivity compared to the Signature Prep Kit and may provide more robust data. In this study, we evaluated the MainstAY kit compared to CE data previously collected from challenging samples using GlobalFiler (Thermo Fisher) and Investigator 24plex QS (QIAGEN) kits. A sensitivity study consisted of varying concentrations of control DNA between 1ng – 8pg.

Results showed that the MainstAY kit had similar or improved recovery compared to the capillary electrophoresis methods for the challenging samples. Overall, quality testing with the MainstAY chemistry demonstrated the chemistry to be highly sensitive and reproducible, with consistent results observed in four repetitions of 12 samples, including controls. Dropout was seen starting with input DNA of 31pg, but approximately 50% of loci were still recovered at 8pg. With challenging samples, the NGS method improved or obtained comparable results to CE in 73% of samples. The most extreme example of recovery improvement was 4% from GlobalFiler to 42% with MainstAY. However, eight samples, including bone, touch, muscle, hair, and saliva extracts, did not obtain a higher recovery percentage of STRs as CE methods. For this reason, these samples will be included for testing with an enhanced PCR buffer for challenging samples.

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1. S.R. Hughes-Stamm; K.J. Ashton; A. Van Daal. Assessment of DNA degradation and the genotyping success of highly degraded samples. *Int. J. Legal Med.*, vol. 125, no. 3, pp. 341–348, 2011, doi: 10.1007/s00414-010-0455-3.
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3. J. Votrubova; A. Ambers; B. Budowle; D. Vanek. Comparison of standard capillary electrophoresis based genotyping method and ForenSeq DNA Signature Prep kit (Illumina) on a set of challenging samples. *Forensic Sci. Int. Genet. Suppl. Ser.*, vol. 6, no. September, pp. e140–e142, 2017, doi: 10.1016/j.fsigss.2017.09.027.

### MiSeq® FGx™, Next Generation Sequencing (NGS), Degraded DNA

## B174 A Modified ForenSeq™ DNA Signature Prep Kit Protocol for Increased Allele Recovery From Low DNA Quantities

Sana Enke, BS, NBACC, Fort Detrick, MD; Kimberly Eskey, MS, NBACC, Fort Detrick, MD; Tracy Ferguson, MS, NBACC, Fort Detrick, MD; Rebecca Just, PhD\*, NBACC, Fort Detrick, MD

**Learning Overview:** After attending this presentation, attendees will understand how optimizing post-PCR steps of a next generation sequencing protocol can recover additional alleles amplified using a human DNA typing assay.

**Impact Statement:** This presentation will provide the forensic science community with a ForenSeq Signature Prep methodology that can help to obtain probative information from low DNA input samples and minor contributors to mixtures.

The next generation sequencing (NGS) based ForenSeq™ DNA Signature Prep kit (Verogen, Inc.) using DNA Primer Mix B targets 58 total autosomal, Y-chromosome and X-chromosome short tandem repeat (STR) markers, plus identity, ancestry and phenotype-informative SNPs in a single multiplex PCR reaction. Similar to traditional size-based STR typing assays, the recommended DNA input for PCR for the Signature Prep kit is 1 ng, though developmental validation indicates complete profiles can potentially be recovered down to 62.5 pg input DNA using the manufacturer's protocol.<sup>1</sup>

To improve the likelihood of recovering probative results from very low template single-source samples and minor contributors in mixtures, we have developed a modified protocol for the Signature Prep kit. The laboratory portions of the modified protocol incorporate multiple post-PCR changes, including: concentration of PCR products at the post-indexing purification step; replacement of the manufacturer's bead-based normalization with equimolar pooling; an additional purification step following library pooling; use of freshly diluted NaOH in place of kit reagent HP3 for consistency in denaturing; addition of HCl to account for a higher concentration of NaOH and fully neutralize the denaturing reaction; and sequencing from loading concentrations ranging from 12-20 pM.<sup>2,3</sup> The modified protocol also utilizes optimized data analysis parameters that aim to retain authentic low-level alleles in mixtures (via a low 1.7% dynamic analytical threshold for most loci), while still filtering error sequences (noise) and stutter (when desired).

This presentation will include empirical data demonstrating the efficacy of the modified protocol as compared to the standard (manufacturer's) protocol for the Signature Prep kit for the recovery of alleles from DNA quantities equal to or less than 50 pg input DNA. In addition, the presentation will demonstrate the value of the increased information recovery on the interpretation of mixtures. The modified protocol produces high quality data from both the assay target template quantity of 1 ng and low template samples, and helps to realize the potential of increased sensitivity to low quantities of DNA using NGS technologies.

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### Next Generation Sequencing (NGS), Low Template Samples, Mixtures

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**B175 Predicting Short Tandem Repeat (STR) Genotypes From Identity-Informative Single Nucleotide Polymorphisms (SNPs) Across Three Ancestry Population Groups**

*Anjalika Balasuriya, HBS, University of Toronto, Mississauga, Ontario, Canada; Nicole Novroski, PhD\*, University of Toronto, Mississauga, Ontario, Canada; Frank Wendt, PhD, Yale University, New Haven, CT*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of a novel approach to genotyping highly compromised/degraded human remains.

**Impact Statement:** This presentation will impact the forensic science community by presenting the use of identity-informative single nucleotide polymorphisms (iiSNPs) genotypes to statistically predict genotypes at short tandem repeat (STR) loci across the genome.

In forensic investigations, highly heterozygous STRs are the gold-standard genetic tool for attributing the source of biological evidence to a suspect. STR genotyping is considerably more difficult when studying compromised evidentiary substrates, skeletonized human remains, and other historical biologicals due to the degraded nature of available DNA.<sup>1,2</sup> Furthermore, once the average DNA fragment length is reduced to sizes smaller than 300 bp, the template DNA may no longer be suitable for current STR typing methods which rely on the amplification of STR markers by polymerase chain reaction (PCR).<sup>3</sup> When processing degraded DNA, numerous PCR amplification issues can occur, such as heterozygous peak imbalance, allelic drop-out, and artifact signals, all contributing to an incomplete and/or low-quality DNA profile.<sup>3</sup>

Relative to STRs, single nucleotide polymorphisms (SNPs), which represent single base changes, can be genotyped using smaller sections of DNA and are not subject to PCR artifacts. Therefore, in suitable numbers, SNPs may be comparably powerful source attribution loci. As SNPs require nearly triple the loci to match STR match statistic power, their applicability to degraded DNA is still limited. In other words, it remains difficult to successfully genotype a full iiSNP profile.<sup>4</sup> Utilizing samples from the 1000 Genomes Project, genotypes of 94 iiSNPs from the ForenSeq™ DNA Signature Prep Kit (Verogen, San Diego, CA, USA) were used to statistically predict genotypes at STR loci across the genome. We report relatively low accuracy of STR genotypes derived from SNP data and discuss the limitations of predicting highly polymorphic STR genotypes for source attribution statistics.

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**Forensic Genetics, Single Nucleotide Polymorphisms, Short Tandem Repeats**

## **B176 The Development of an Enhanced Buffer to Overcome Polymerase Chain Reaction (PCR) Inhibition for DNA Typing by Targeted Next Generation Sequencing (NGS)**

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**Learning Overview:** After attending this presentation, attendees will gain information on an option to overcome PCR inhibition in challenging casework samples for DNA typing by targeted Next Generation Sequencing.

**Impact Statement:** This presentation will impact the forensic science community by presenting studies for improved DNA typing of challenging samples using targeted Next Generation Sequencing with highly multiplexed STR and SNP amplicons.

Targeted Next Generation Sequencing (NGS) or massively parallel sequencing (MPS) has recently emerged as an efficient approach in forensic DNA analysis to profile both short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers at a relatively high plexity.<sup>1,2</sup> Yet, current NGS-based approaches, unlike capillary electrophoresis-based ones (CE), are susceptible to PCR inhibitors.<sup>3,4</sup> Common inhibitors in forensic samples including humic acid (from soil) and hematin (from blood) have been shown to negatively impact library preparation PCR amplification yield and consequently lead to lower sequencing reads and allele detection rate. The sequencing results also show that larger amplicons tend to drop out in the presence of inhibitors.

Approaches to overcome inhibition in forensic samples include dilution or further purification of samples, adjusting polymerase, bovine serum albumin (BSA), and magnesium concentration, and the use of smaller amplicons in PCR reactions. Addition of 10 ug BSA alone to the initial PCR reaction in ForenSeq DNA Signature Prep Kit has been shown to improve inhibitor tolerance significantly.<sup>4</sup> However, developing NGS assays for DNA typing encounters challenges not only on PCR inhibition, but also with PCR and sequencing fidelity. Additional challenges for ForenSeq DNA Signature Prep Kit are to target both large and small markers at a high plexity. Here an enhanced PCR buffer (ePCR1) utilizing PCR additives, such as BSA, PEG, glycerol, and DMSO with the high-fidelity polymerase was developed and verified.

The verification of ePCR1 was performed following the revised guidelines for developmental validation from the Scientific Working Group on DNA Analysis Methods (SWGDM, December 2016), with addendum on STR typing to address NGS (April 2019). Two multiplexes of markers were tested with ePCR1: 1) DNA Primer Mix B (DPMB) targets 27 autosomal STRs (aSTRs), 24 Y-STRs, 7 X-STRs, and 172 SNPs; 2) DNA Primer Mix C (DPMC) targets 27 aSTRs and 25 Y-STRs.

Comparing to the original PCR1 buffer, the inhibitor tolerance with ePCR1 is improved by 50-fold for humic acid, 3.3-fold for hematin, and 8-fold for tannic acid for DPMC. In the absence of inhibitors, metrics such as allele balance, locus coverage, and sequencing noise remain comparable between PCR1 and ePCR1 buffers. Stutter, however, is slightly elevated with ePCR1 buffer compared to PCR1 buffer. Other studies such as species-specificity, sensitivity, accuracy and precision, stability, and casework-type samples such as bone extracts will be discussed. PCR1 buffer is suitable to profile regular DNA samples while ePCR1 buffer is an option for challenging, inhibited samples for targeted NGS assays.

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4. Sidstedt, M.; Steffen, C.R.; Kiesler, K.M.; Vallone, P.M.; Rådström, P.; Hedman, J. The impact of common PCR inhibitors on forensic MPS analysis. *Forensic Sci Int Genet.* 2019, 40:182-191.

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### **DNA Typing, PCR Inhibition, Next Generation Sequencing (NGS)**

## **B177 Evaluating Library Preparation Improvements of Short Tandem Repeats (STRs) Using the PowerSeq™ 46GY System for Massively Parallel Sequencing (MPS)**

*Kyleen Elwick, PhD\*, Federal Bureau of Investigation, Quantico, VA; Patrick Rydzak, PhD, Federal Bureau of Investigation, Quantico, VA; James Robertson, PhD, Federal Bureau of Investigation, Quantico, VA*

**Learning Overview:** After attending this presentation, attendees will gain an understanding of how different library preparation methods, purification beads, and quantification kits affect the results of the PowerSeq™ 46GY System.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of testing a variety of library preparation methods to obtain the best quality results for forensic-type samples using a commercial STR panel.

Massively parallel sequencing (MPS) provides an alternative to Sanger sequencing and CE-based analysis for DNA typing. It provides increased sequencing throughput and sensitivity capabilities, as well as the ability to sequence multiple markers simultaneously (i.e., SNPs, STRs). This project evaluated the PowerSeq™ 46GY System, containing 22 autosomal loci, 23 Y-STR loci, and Amelogenin, using male and female control DNA and casework-type bone and hair samples.

The goal of this study was to determine which library preparation kit, bead purification method, and quantitative PCR (qPCR) kit provided the highest coverage and best quality results for these samples with the PowerSeq™ 46GY System. The male and female control samples (DNA input amounts from 6.3pg to 1ng) and casework-type samples were amplified with the PowerSeq™ 46GY System and prepared with either the TruSeq® DNA-PCR Free HT Library Preparation Kit (TruSeq®), or the KAPA HyperPrep Kit (KAPA). The library preparation kits were evaluated unmodified, as well as by substituting Agencourt® AMPure® XP beads for the beads of each respective kit. Two qPCR kits were also evaluated with all libraries including the PowerSeq™ Quant MS System (PowerSeq™) and the KAPA Library Quantification Kit (KAPA). The prepared libraries were sequenced using the MiSeq® FGx and data were analyzed with Battelle ExactID® software.

The first experiment in this study evaluated the TruSeq® kit with TruSeq® Sample Preparation Beads using five concentrations of male and female control DNA. The KAPA kit resulted in quantities 2.6-8.7X higher than the PowerSeq™ kit. Post sequencing, coverage was high for all DNA input concentrations, averaging ~3000X-5500X coverage. Samples with a total DNA input of 0.5ng demonstrated the highest read depth. Furthermore, all loci amplified and demonstrated high coverage for each sample.

The second experiment in this study evaluated the KAPA kit with KAPA Purification Beads using five concentrations of male and female control DNA. Again, KAPA quantified libraries were 6-9.2X higher than the PowerSeq™ kit. The sequencing results demonstrated an increase in coverage as the DNA input concentration increased. Average coverage ranged from ~150X-675X, with 1ng samples producing significantly higher ( $p < 0.05$ ) coverage than all other DNA concentrations. In addition, unequal amplification was observed among loci.

The third experiment in this study evaluated the TruSeq® kit with AMPure® beads using five concentrations of male and female control DNA. The PowerSeq™ and KAPA kits resulted in a large range of quantification values for all libraries. Moreover, the KAPA quantified libraries were 10-20X larger than the PowerSeq™ libraries. Coverage ranged from ~4500X-6700X with the highest coverage at 0.25ng of DNA input. Additionally, all STR loci amplified and demonstrated high coverage.

The final experiment in this study evaluated casework-type samples ( $n=12$  bone/hair) using the TruSeq® kit with AMPure® beads in place of kit-provided beads. Bone and hair quantities prior to amplification ranged from 0.01ng - 0.51ng DNA input. Using the PowerSeq™ and KAPA library quantification kits, hair samples resulted in quantities up to 7X higher than bone samples. Average sample coverage was ~2800X and coverage ranged from ~1200X (0.01ng) to ~4300X (0.42ng). Additionally, all loci amplified and demonstrated coverage over 1000X using casework-type samples.

Overall, the samples amplified with the PowerSeq™ 46GY System produced the highest quality results when libraries were prepared using the TruSeq® library preparation kit and purified with AMPure® beads.

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### **Massively Parallel Sequencing, STR, Mitochondrial DNA**



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## **B178 Forensic Autosomal Short Tandem Repeat (STR) Profiling Using the Promega® PowerSeq™ Kit on Oxford Nanopore® Technologies' MinION Device**

*Courtney Hall, MS, UNTHSC, Fort Worth, TX; Rupesh Kesharwani PhD, Baylor College of Medicine, Houston, TX; Nicole Phillips, PhD, UNTHSC, Fort Worth, TX; John Planz, PhD, UNTHSC, Fort Worth, TX; Fritz Sedlazeck, PhD, Baylor College of Medicine, Houston, TX; Roxanne Zascavage, PhD\*, UNTHSC, Fort Worth, TX and UTA, Arlington, TX*

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**Learning Overview:** Attendees will learn about a successful approach to forensic STR profiling using Promega PowerSeq46 kit on the MinION device when data is assessed with our novel program; STRspy.

**Impact Statement:** This presentation will demonstrate that nanopore sequencing platforms are capable of revealing an additional level of variation in and around STR loci with sufficient read coverage. As the first method to successfully profile the entire panel of autosomal STRs amplified by a commercially available kit using an ONT platform, STRspy significantly increases the feasibility of using Nanopore sequencing in forensic applications.

The high variability characteristic of short tandem repeat (STR) markers is harnessed for human identification in forensic investigations. Despite the power and reliability of current typing techniques, sequence-level information both within and around STRs are masked in the length-based profiles generated. Forensic STR profiling using next generation sequencing (NGS) has therefore gained attention as an alternative to traditional capillary electrophoresis (CE) approaches.

In this proof-of-principle study, we evaluate the forensic applicability of the newest and smallest NGS platform available, the Oxford Nanopore Technologies (ONT) MinION device. Although nanopore sequencing on the handheld MinION offers numerous advantages, including on-site sample processing, the relatively high error rate and lack of forensic-specific analysis software has prevented accurate profiling across STR panels in previous studies. Here we present STRspy, a streamlined method capable of producing length- and sequence-based alleles designations from noisy, long-read data. To demonstrate the capabilities of STRspy, seven reference samples (female: n = 2; male: n = 5) were amplified using the Promega PowerSeq 46GY System and sequenced on the ONT MinION device in triplicate. Basecalled reads were processed with STRspy using a custom STR database containing alleles reported in the STRSeq BioProject NIST 1036 dataset. Resultant STR allele designations and flanking region SNP calls were compared to the manufacturer-validated genotypes for each sample. STRspy generated robust and reliable genotypes across all autosomal STR loci using 500pg of input DNA amplified with 30 PCR cycles, achieving 100% concordance based on both length and sequence. Furthermore, we were able to identify flanking region SNPs with >90% accuracy.

These results demonstrate that nanopore sequencing platforms are capable of revealing an additional level of variation in and around STR loci with sufficient read coverage. As the first method to successfully profile the entire panel of autosomal STRs amplified by a commercially available kit using an ONT platform, STRspy significantly increases the feasibility of using Nanopore sequencing in forensic applications.

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### **Forensic DNA Analysis, STR, Nanopore® Sequencing**

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## **B179 GeoFOR: An Innovative Geographic Information Systems (GIS) -Based Application and Accumulated Degree Days (ADD) Calculator**

*Cristina Tica, PhD\*, Clemson University, Clemson, SC; Katherine Weisensee, PhD, Clemson University, Clemson, SC; Patrick Clafin, Clemson University, Clemson, SC; Patricia Carbajales-Dale, MS, Clemson University, Clemson, SC*

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**Learning Overview:** Attendees will learn about the newly developed, Geographic Information Systems (GIS)-based application created with the aim of generating a large and representative database of forensic cases. The main goal for collecting a robust dataset is to integrate it with embedded georeferenced information and other curated environmental data, in order to develop accurate models, with known error rates, for calculating time since death.

**Impact Statement:** This presentation will show how all death investigators, coroners, coroner deputies, medical examiners, forensic educators, and any members of the forensic science community studying and recording deaths can contribute to this project. As the application is already available for use by investigators from across the country and internationally (~40 users thus far), it brings the forensic community together in the creation of an extensive reference database (~ 200 cases and growing) of decomposition and environmental variables, reflective of the realities of forensic casework.

GIS software provides an ideal system from which environmental variables from a local area can be easily obtained from available databases and overlaid on the location of the scene. The current application prototype was developed in Survey123 for ArcGIS software. ArcGIS provides a secure system that is currently utilized by multiple industries and governmental organizations for securely storing sensitive data.

The GeoFOR application provides a framework for data acquisition which minimizes interobserver error and maximizes efficiency. In order to encourage practitioners to submit data from casework, the application has a built-in calculator that provides practitioners with immediate benefits: it calculates the accumulated degree days based on the location the remains are recovered. Accumulated degree days is a commonly used variable for many of the available methods for estimating the postmortem interval (PMI). However, it is a time-consuming process for forensic investigators to recover information from nearby weather stations so as to calculate accumulated degree days. Using the GeoFOR application, the investigator inputs the information about the case and the application automatically compiles information from the National Oceanic and Atmospheric Administration (NOAA), with weather stations available worldwide, in order to quickly determine the accumulated degree days. This addition to the application uses spatial analysis techniques to locate the nearest weather station. Using a Python script, the application gathers historical weather information from the station's RSS feed and calculates average temperature until it reaches the accumulated degree days needed. By providing investigators with this information in a simpler and faster format compared to currently available methods, it provides an immediate incentive for practitioners to contribute their data beyond the long-term goal for improving time since death estimates.

Many statistical and machine learning methods exist which could significantly improve determinations of time since death; however, all these methods depend on the availability of known reference or training sets to develop new methods. In the absence of improved data, it will be difficult to develop improved methods for estimating time since death. This reference set must reflect the reality of forensic casework in order to be applicable to future cases. This means it needs to be geographically diverse and encompass the various scenarios in which human remains are discovered in forensic casework. The goal of this project is to develop this reference data set which will be used to train new models, validate methods, and suggest future experimental studies to refine the understanding of decomposition.

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### **Postmortem Interval (PMI), Decomposition, Taphonomy**

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## B180 The Billey **Joe** Johnson Homicide

*Bryan Burnett, MSc\*, Meixa Tech, Cardiff, CA*

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**Learning Overview:** After attending this presentation attendees will understand that for unexpected deaths where the official manner of death is disputed by any party, an examination of the evidence by an independent, experienced death scene analyst should be required.

**Impact Statement:** Local law enforcement and district attorneys for both the county and state of Mississippi ignored the evidence of homicide and declared the victim committed suicide without any supportive physical evidence.

In the early morning of December 8, 2008, seventeen-year-old Billey Joe Johnson allegedly committed suicide by discharge of his 12-gauge shotgun to the left side of his head at the ear. The death occurred in George County, a rural county in southern Mississippi, USA. Johnson was beside his truck on the driveway to a small business park at the time of his death. According to the only witness to the shooting, a police officer, “I walked back to my vehicle got down my glasses to read the driver’s license [of the victim] and I heard a gunshot and glass break. I looked up, the black male fell on the ground and the gun he had in his hand fell on top of him.”

Johnson was officially determined to have committed suicide but the death scene, processed as a homicide, indicated otherwise. The manner of death was changed to accident by a Mississippi Grand Jury in a decision published February 2009. In April 2011 a letter from the United States Department of Justice, Civil Rights Division to the parents of Billey Johnson supported the suicide death.

Images of the scene were taken by five digital cameras at different times over an eleven-hour span. All of these images appeared to have relatively accurate timestamps in their metadata files.

Blowback from the penetrating 12-gauge shotgun discharge to the victim’s head was substantial, propelling tissue as much as 14.3 m (47 feet) from the victim. The victim’s head was within 30 cm (1 foot) of the interior truck door’s window when the shotgun discharged, a position not possible for either suicide or accident scenarios. The victim was not the shooter by a shotgun that was not his.

The bloodstains show the body was moved twice following the discharge of the shotgun and prior to law enforcement, other than the police officer witness, arriving on scene. The shotgun was placed on the body to stage suicide. A scene processor or a person with access to the scene continued the staging of the scene by placing a handwritten love note (3 x 5 inch card), apparently to the victim, on the dash of his vehicle and moving the card twice during the scene processing. The card was not put into evidence. It was either removed from the truck’s dashboard prior to the victim’s family taking possession of the truck or it was left for the family.

There were multiple assailants involved in Johnson’s death. The person who fired the shotgun (not the victim’s shotgun) was standing near the exterior of the open driver side door. He thrust the shotgun barrel through the closed window breaking it. Two or more persons held the victim and positioned him to be shot near the interior side of the open truck door.

Lynching: “...a killing by a group. It is often used to characterize informal public executions by a mob in order to punish ... or intimidate.” - Wikipedia. Billey Joe Johnson was lynched.

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### Crime Scene Reconstruction, Staged Suicide, Image Metadata

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## **B181 The Application of Artificial Intelligence (AI) Image Analysis Techniques to Bloodstain Pattern Analysis**

*James Creecy, PhD\*, University of Central Oklahoma, Edmond, OK; Jicheng Fu, PhD, University of Central Oklahoma, Edmond, OK; Craig Gravel, MA, University of Central Oklahoma, Edmond, OK*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the uses of artificial intelligence and the role it can play in assisting a trained bloodstain pattern analyst.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence on the application of artificial intelligence to the field of bloodstain pattern analysis.

The forensic discipline of Bloodstain Pattern Analysis (BPA) has played a crucial role in the reconstruction of countless crime scenes. The shape, size, distribution, and location of bloodstains at a crime scene have aided trained analysts in locating potential murder weapons, estimating the origin of attacks, and determining if a body had been moved or relocated from the original crime scene. Despite well-established legal precedent, there continue to be aspects of BPA that can be improved upon. To date, BPA relies primarily on manual methods of analysis to process a crime scene. As with most forensic disciplines, BPA analysts require rigorous training on the use and application of these manual methods before applying them at a crime scene. It is therefore imperative to have forensic analysts who can accurately produce consistent and reliable results. However, human error has been known to occur under challenging case scenarios, and analysts have been known to interpret the same body of evidence differently. Commonly, errors in identifying blood spatter evidence arise when the crime scene has large amounts of bloodstains which can yield less information during analysis. Based on the overwhelming success of artificial intelligence (AI) image analysis in other areas of science and medicine, it is logical to infer that automated and computer-assisted analysis methods can be employed successfully to analyze bloodstain patterns. The goal of this study was to utilize AI algorithms to assist the analyst in the analysis of bloodstain patterns. To achieve this goal, a few AI algorithms were employed to estimate the angle of impact for a large data set of controlled spatter pattern samples. This study focused on the analysis of photos taken from a single impact angle as the primary input data. Bloodstain patterns were experimentally constructed using controlled conditions, and a single variable was altered at a time. This study determined that the AI-assisted approach accurately assigned the correct angle of impact for 78.64% of all data analyzed. Interestingly, the angle range that the AI was unable to resolve accurately, 80°-60°, is the same angle range that the manual methods of analysis struggle to resolve. In conclusion, AI-based analysis of bloodstain patterns is a promising area of study to improve the forensic discipline of bloodstain pattern analysis.

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**Artificial Intelligence, Bloodstain Pattern Analysis, Image-Processing Method**

## B182 Determining the Minimum Size of Soil Samples For Forensic Geological Analysis

*Brittany Claassen, BS\*, Cedar Crest College, Allentown, PA; Emma Redman, Cedar Crest College, Allentown, PA; Ted Schwartz, MS, Westchester County Forensic Laboratory, Valhalla, NY; Thomas Brettell, PhD, Cedar Crest College, Allentown, PA; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA*

**Learning Overview:** Attendees will learn that the minimum amount of soil needed for result reliability varies according to method and soil type.

**Impact Statement:** This research will impact the forensic science community by contributing to the understanding of the minimum soil techniques before divergent and spurious results occur.

**Goal of Project:** The goal of this project is to analyze and determine the sensitivity of several common soil techniques and to determine the minimum sample needed for robustness of method.

Although not widely practiced in most forensic laboratories, numerous case studies exist in literature showing the evidentiary value of the forensic comparative analysis of soil.<sup>1</sup> Soil in a forensic context can be of limited size and although numerous comparative techniques exist, the minimum sample size required for reproducible and accurate data in each of these techniques is not well known. In fact, determining the minimum sample size for visual color determination of soil samples has been identified as an OSAC research need. In this study, 15 surface layer soil samples from different geologic areas were collected and homogenized for analysis. Various techniques including digital color determination, particle size distribution, d-space ordering by x-ray diffraction (XRD), and loss on ignition (organic content) were applied to sample sizes ranging from 2 to 0.25 grams. Differences in color between samples was measured using the Nix Color Pro QC Sensor and calculated using Delta E. It was found that color differences between samples and sample sizes was highly dependent on particle size components within samples. Locations that consisted of higher portions of silt and clay fractions reported low Delta E values between sample sizes and among sample sizes, compared to samples with higher coarse fractions which reported low Delta E values between sizes and among samples sizes. Descriptive statistics were utilized on particle size distribution and loss on ignition data. The use of correlation analysis and Kolmogorov–Smirnov D statistic suggests that samples below 1 gram begin to show diverging results. Analysis of variance performed on loss on ignition determined that samples with lower carbon content lost showed statistical differences between sample sizes. In addition, confidence intervals indicate that 0.25-gram samples do not often overlap with other sample sizes within each location. D-space ordering via x-ray diffraction was done on 2- and 0.25-gram samples of each location and it was determined that both were visually different. To determine where the differences in sample sizes begin to diverge via XRD, further analysis will be performed on 1- and 0.5-gram samples. Overall, based on preliminary data findings and analysis, samples smaller than 0.5 grams begin to give divergent results compared to larger sample sizes.

### Reference(s):

- <sup>1</sup> Petraco, N.; Kubic, T.A.; Petraco, N.D.K. (2008). Case studies in forensic soil examinations. *Forensic Science International*, 178(2–3), 23–27. <https://doi.org/10.1016/j.forsciint.2008.03.008>.

### Comparative Soil Examination, Sample Size, Sensitivity

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## **B183 The Development of a Technique for the Forensic Identification of Carrion Beetles**

*Amy Osborne, BS\*, University at Albany, SUNY, Albany, NY; Jennifer Rosati, PhD, John Jay College of Criminal Justice, New York, NY; Rabi Musah, PhD, University at Albany, SUNY, Albany, NY*

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**Learning Overview:** Attendees can expect to learn about the use of DART®-HRMS for the generation of unique insect species-specific metabolome profiles, and how statistical analysis can be applied to these profiles to enable insect species identification that is useful for postmortem interval determination.

**Impact Statement:** The use of metabolome profiles as unique chemical fingerprints that enable differentiation between species of insects will be presented. Attendees will also learn about the application of statistical analysis for processing of insect chemical profiles for differentiation and classification, as well as about methods for handling specimens.

One of the most important aspects of a death investigation is estimation of time since death or postmortem interval (PMI). This can be difficult to assess for remains that have advanced to such a state of decay that traditional methods for determining PMI are no longer applicable. One approach that can be employed in such cases is the use of carrion insects found on or near the body, as there is a well-established correlation between a given stage of decomposition, and the insect species that colonize the remains. Since the species-dependent timeline associated with insect progression through various life stages is well established, knowledge of the species of the retrieved entomological evidence can be used to calculate PMI. For this reason, accurate species identification is critical. However, while this may seem straightforward, it is often a time-consuming, resource-intensive process that requires high levels of specialized expertise and laboratory facilities, among other elements. This is because traditional insect species identification is accomplished by rearing collected immature life forms to maturity so that positive identification based on the gross physical features of the adult can be achieved. Furthermore, the insects most likely to be found colonizing remains that are in the advanced stages of decay are beetles. Yet, there is a limited amount of research on methods that can be used to quickly identify beetle species.

It is demonstrated here that chemometric processing of direct analysis in real time mass spectrometric data acquired from analysis of insects can be used to rapidly accomplish species identification. In order to mimic field collection practices, all specimens were suspended in 70% aqueous ethanol prior to analysis by DART®-HRMS. It was established that consistent results were obtained when samples were suspended in aqueous ethanol for at least 24 hours, and that suspending the samples for more than 24 hours did not result in added advantage, nor was an advantage seen in rehydrating dried insect specimens with steam prior to placing them in suspension. Furthermore, it was determined that insect specimens could be resuspended in aqueous ethanol after several months in dry storage while still yielding results similar to what would be obtained from analysis of fresh specimens. Overall, the results showed that the ethanol suspensions of each species exhibited a unique chemical fingerprint, and that these fingerprints were consistent for members of the same species but different between species. The application of Kernel Discriminate Analysis (KDA) to the data revealed that clear differentiation was possible between insect species based on the chemical fingerprints generated by DART®-HRMS analysis of insect-ethanol suspensions. Furthermore, the separation occurs even between insects belonging to members of the same family, such as the Silphidae family. Thus, the use of DART®-HRMS and statistical analysis to differentiate between species of insects can circumvent some of the challenges faced when dealing with entomological evidence, thereby increasing the utility of this evidence.

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**Forensic Entomology, DART®-HRMS, Chemometric**

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## **B184 Human Scent Biometrics: A Pilot Study in Source-Origin Determination**

*Vidia A. Gokool, MS\**, International Forensic Research Institute, Florida International University, Miami, FL; *Howard K. Holness, PhD*, International Forensic Research Institute, Florida International University, Miami, FL; *Kenneth G. Furton PhD*, International Forensic Research Institute, Florida International University, Miami, FL

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**Learning Overview:** After attending this presentation, attendees will understand the application of a combined targeted and untargeted approach to gas chromatography-mass spectrometry (GC-MS) data analysis as applied to the interpretation of profile variability in same-donor human hand odor samples.

**Impact Statement:** This presentation will impact the forensic science community by sharing newly cultivated fundamental knowledge regarding the variability of human hand odor and equipping attendees with techniques for extrapolating similarities in GC-MS profiles through a pattern recognition approach.

Human scent is commonly implemented as an individualizing feature used to associate a suspect with an object, location, or path of travel. Human scent evidence is commonly identified by and utilized in conjunction with human scent detection canines. Human scent detection canines are employed to follow a scent trail left by a person of interest or to associate a questioned item with one of known origin.

Previous studies in the field of human scent analysis have worked to explain the procedures carried out by these canines when associations are performed; however, these works have largely focused on describing the differences between separately sourced samples. The presented work builds upon previous research in the field while providing insight into the under-evaluated fundamental theory regarding the intra-subject variability of human scent. The presented work evaluates the volatile organic compounds (VOCs) present in the headspace of human hand odor samples collected from 8 donors over variable time periods ranging from 5-weeks to 1-year. These profiles were collected over the course of multiple weeks, utilizing headspace-solid phase microextraction- gas chromatography-mass spectrometry (HS-SPME-GC-MS) to capture and analyze the gaseous organic compounds present in the equilibrated sample headspace. With the headspace composition serving as a representative depiction of the donor's hand odor composition, both targeted and untargeted approaches to profile interpretation and association were investigated.

This research evaluates a combined approach to analyzing VOCs, incorporating Spearman's rank correlation, 3-D covariance, and automated peak matching as chemometric techniques for analyzing 3-D and 2-D components of human hand odor profiles. This combined approach provides a mechanism for predicting the source-origin of human scent samples collected using HS-SPME-GC-MS. Intra-subject variations in human scent, including inter-day changes, are explored. A discussion will be lead on the observed diversity in human scent, its impact on the developed technique performance, and the plans for further refining of the developed methodology.

This study reflects an active effort towards the creation of a model for the formal discrimination of human scent profiles collected using GC-MS. Further application of the advances made in this study will aid in the understanding of same-donor human scent similarity and inform thresholds when discriminating between different-donor samples. Additionally, this work will provide greater context to canine performed human scent associations while continuing to build toward a quantitative laboratory test for confirmation of in-field, canine performed human scent associations.

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### **Human Scent, Chemometric, HS/SPME-GC/MS**

## B185 Volatile Organic Compounds (VOCs) Produced by Bacteria Associated With Decomposition

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**Learning Overview:** Attendants can expect to learn how the microbiome and VOC profile are connected throughout the complex process of decomposition.

**Impact Statement:** This presentation will provide the potential origin of VOCs observed throughout decomposition, giving further insight to impact of microorganisms on the process, and improving techniques for locating remains.

Microorganisms play an important role in decomposition and are known to produce volatile organic compounds (VOCs) that contribute to the odor of decomposition. Although microorganisms and VOCs have been studied independently regarding decomposition, few studies have linked the two subject matters. As the number of decomposition studies increases, a clearer picture of the temporal evolution of VOC profiles is emerging. However, identifying the origin(s) of specific VOCs remains elusive (i.e., microbial processes, general chemical decomposition, insects, environmental effects). The volatile organic compounds produced by specific species of bacteria associated with decomposition were characterized in order to determine origins of VOCs detected during decomposition. In the present study, the volatile organic compounds produced by specific species of bacteria associated with decomposition were characterized. Overall, researching the VOCs produced by bacteria will help to understand the complex process of decomposition. It also helps improve techniques used to search for remains in missing person cases or natural disasters, such as through improved training of cadaver-detection canines or the creation of VOC detection devices. Microbial communities were sampled at various time points during decomposition of a swine placed in an indoor enclosure and sequenced using NextGen Illumina sequencing. Because the indoor enclosure inhibited insect activity and colonization by extrinsic microorganisms, the VOC profile likely reflects the intrinsic microbial community and autolysis. Over 1,000 taxa at the genus level were identified and over 600 taxa at the species level. Prevalent phylum includes Bacteroidetes, Firmicutes, Tenericutes, and Spirochaetes, which are seen in other swine decomposition studies.<sup>1</sup> Further analysis of the swine's microbiome succession is discussed with an emphasis on the potential correlation of the microbiome succession and decomposition as seen in other studies like Hyde et al. and Metcalf et al.<sup>2,3</sup> Based on the relative abundance of sequenced reads, *Alcaligenes faecalis*, *Lysinibacillus fusiformis*, and *Lactobacillus gasseri* were selected. They were independently cultured in headspace vials on a modified chopped meat medium created from ground pork broth and sheep blood, to provide similar nutrients to the decomposing swine. Solid Phase Microextraction (SPME) fibers were used to sample the VOCs produced by the bacterial species. The VOC profiles produced by each bacteria species are compared with the overall VOC profile collected from the same decomposing swine to distinguish the VOCs associated with bacterial decomposition from those originating from the general break-down process. *A. faecalis* produced VOCs categorized as sulfides and carboxylic acids. *L. fusiformis* generated sulfides and hydrocarbons, while *L. gasseri* was seen to produce just one VOC. However, this may be due to the nutrient source and growth conditions. To investigate how interactions between species could affect VOC production, *A. faecalis* and *L. fusiformis* were cultured together. Different VOCs versus in the independent cultures were produced, like nitrogen-containing compounds that have been seen in other decomposition studies.<sup>4</sup> This may support that species interactions can have an extraordinary effect on VOC production.

### Reference(s):

1. Pascual, J.; von Hoermann, C.; Rottler-Hoermann, A.M.; Nevo, O.; Geppert, A.; Sikorski, J., et al. Function of bacterial community dynamics in the formation of cadaveric semiochemicals during in situ carcass decomposition. *Environmental microbiology*. 2017;19(8):3310-22.
2. Hyde, E.R.; Haarmann, D.P.; Lynne, A.M.; Bucheli, S.R.; Petrosino, J.F. The living dead: bacterial community structure of a cadaver at the onset and end of the bloat stage of decomposition. *PloS one*. 2013;8(10):e77733.
3. Metcalf, J.L.; Parfrey, L.W.; Gonzalez, A.; Lauber, C.L.; Knights, D.; Ackermann, G., et al. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. *elife*. 2013;2:e01104.
4. Rosier, E.; Cuypers, E.; Dekens, M.; Verplaetse, R.; Develter, W.; Van de Voorde, W., et al. Development and validation of a new TD-GC/MS method and its applicability in the search for human and animal decomposition products. *Analytical and bioanalytical chemistry*. 2014;406(15):3611-9.

### Decomposition, Microbiome, VOCs



## B186 Analyzing the “Cross-Hatch” Patterns of Clear Biaxially Oriented Polypropylene (BOPP) Tapes

Walter Rowe, PhD\*, The George Washington University, Washington, DC

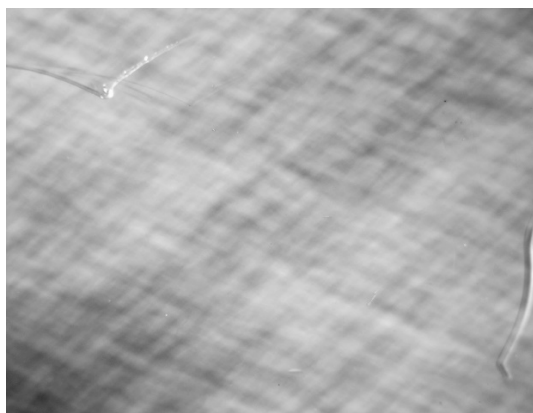
**Learning Overview:** After attending this presentation, attendees will understand how to analyze the “cross-hatch” patterns observed when clear biaxially oriented polypropylene (BOPP) tapes are examined with a polarizing microscope.

**Impact Statement:** This presentation will impact the forensic science community by providing a process for the comparison of “cross-hatch” patterns of questioned and known tape samples.

Clear biaxially oriented polypropylene (BOPP) tapes are widely used pressure-sensitive tapes. These tapes are marketed as mailing tapes, shipping tapes, packaging tapes or storage tapes and are sold at hardware stores, office supply stores, supermarkets, and pharmacies. When examined with a polarizing microscope with crossed polarizing filters, clear BOPP tapes show complex “cross-hatch” patterns when oriented close to extinction. As shown below, the patterns may differ from one tape sample to another.



3M Shipping Tape



Duck EZ Start Heavy Duty Tape

Twenty-four clear BOPP tape samples representing twenty brands were purchased online and at a variety of retail outlets in the Washington, DC, area. The brands included national brands, as well as store brands. Samples of the tapes were placed on standard microscope slides and their “cross-hatch” patterns observed with a polarizing microscope. The patterns were recorded as 8-bit grayscale images using a digital eyepiece camera. The images were pre-processed by application of a diffuse gaussian edge detection filter followed by conversion to binary. These steps preserved the salient features of the “cross-hatch” patterns and removed variations in illumination (vignetting) from most of the patterns.

The binary images were analyzed in two ways.

The fractal dimensions of the binary images were determined using a box-counting algorithm which determined the number  $N(\delta)$  of boxes with edges of length  $\delta$  required to cover completely the white or black areas of a binary image. Generally

$$N(\delta) \sim \delta^{-D}$$

where  $D$  is the box-counting fractal dimension of the image. The box-counting algorithm determines  $N(\delta)$  for a series of values of  $\delta$ . The relationship between  $N(\delta)$  and  $\delta$  can be expressed as a linear equation:

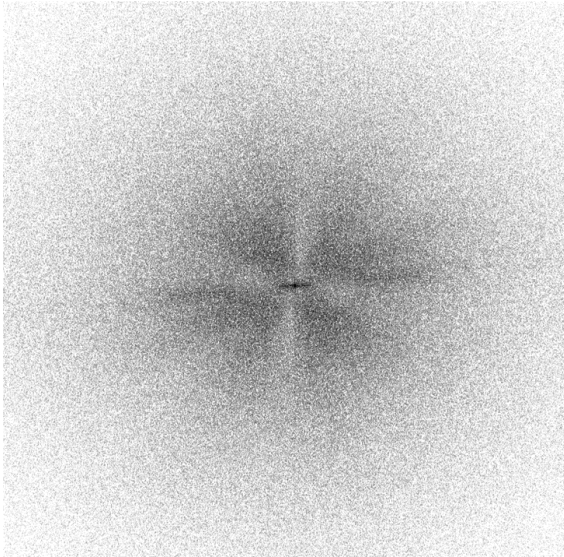
$$\log[N(\delta)] = A - D \log(\delta)$$

Linear regressions were applied to the data from the box-counting algorithm to obtain the best-fit values of  $A$  and  $D$ . The  $A$  and  $D$  values of the tape samples were compared pair-wise using the Student’s  $t$  test.

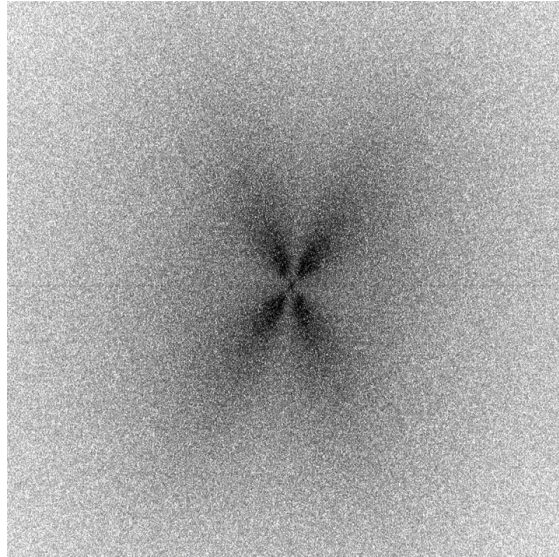
Two dimensional discrete Fourier transforms (2DDFT) were calculated for each binary image. These transforms detect repetitive features in images and have been useful in the forensic analysis of surface texture patterns on sheets of paper created by the fine metal meshes used in paper manufacture.

Analysis of the data for the box-counting fractal dimensions of the binary images of the tape samples revealed that the fractal dimensions  $D$  of the binary images of the tape “cross-hatch” patterns were similar and provided only limited discrimination of tape samples. The intercepts  $A$  provided a greater degree of discrimination than the fractal dimensions. Many of the tape samples had indistinguishable  $A$  and  $D$  values.

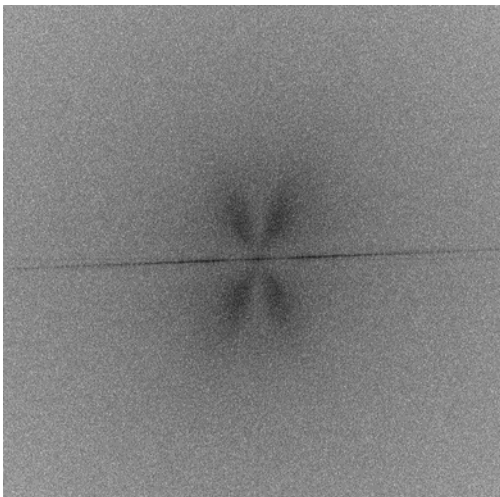
Examination the 2DDFTs of the tape samples revealed that one tape sample had a unique 2DDFT. The remaining 2DDFTs could be placed in three distinct groups, as shown below. The 2DDFTs detected additional subtle repetitive patterns in the “cross-hatch” patterns (e.g. Group 3); they also allowed a more accurate determination of the angles of the “cross-hatch” patterns.



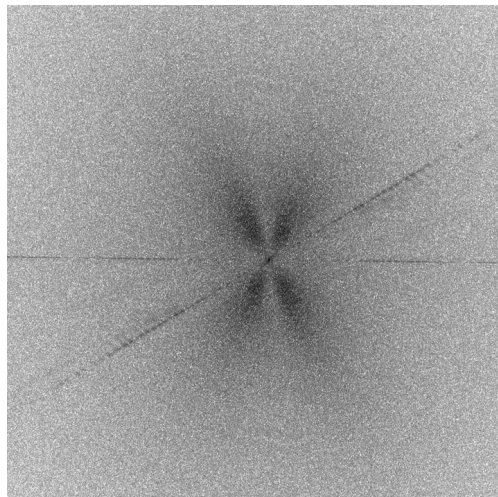
Unique Tape Pattern



Group 1 Fourteen Tapes



Group 2 Seven Tapes



Group 3 Two Tapes

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## Clear Bixially Oriented Polypropylene Tapes, Polarized Light Microscopy, Digital Image Analysis

### **B187 “Leaf” It to DART®-HRMS and Multivariate Statistical Analysis for the Forensic Identification of Illegally Traded Timber**

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**Learning Overview:** Attendees will be introduced to the development of a method to identify illegally traded timber to be used in wildlife forensics laboratories. An understanding of multivariate statistical analysis to aid in the classification will be gained.

**Impact Statement:** This presentation will impact the forensic science community by providing a new and efficient methodology for the identification of illegally traded timber. This approach will contribute to the enhancement of techniques that enable law enforcement to distinguish between endangered wood species at crime scenes.

One of the concerns of wildlife forensics is the identification of endangered species, the trade of which is illegal. The Convention on International Trade of Endangered Species (CITES) was created to address the conservation of imperiled wildlife by controlling their trade. Regulation status is defined by appendices: CITES Appendix I species are threatened with extinction and trade of any kind is outlawed; CITES Appendix II species are threatened in the wild and international trade is controlled to aid in their survival; and CITES Appendix III species are regulated in a particular region. While trade in fauna, including elephant parts (such as tusks), rhinoceros horns, and pangolin scales, are well-known examples of wildlife crimes, there are a host of flora that are also heavily trafficked. *Dalbergia* genus trees serve as a case in point. *Dalbergia* species fall under the Leguminosae family, with most species commonly known as rosewood. Illegal trade of these species is common because they are highly prized for making exclusive furniture, cabinetry, musical instruments and artifacts. Depending on the species, trade is either totally or heavily restricted. A current technique used by law enforcement to differentiate species of wood is direct analysis in real time-high resolution mass spectrometry (DART®-HRMS), coupled with multivariate statistical analysis. Here, the added dimension of wood headspace analysis featuring solid phase microextraction (SPME) was used to generate data to complement that acquired using the conventional wood analysis technique. This could facilitate the development of “stand-off” approaches to the differentiation of wood species based on their volatile profiles. Seventeen *Dalbergia* species, including *D. baronii*, *D. cearensis*, *D. oliveri*, *D. occulta*, *D. madagascariensis*, *D. latifolia*, *D. melanoxydon*, *D. normandii*, *D. purprascens*, *D. retusa*, *D. nigra*, *D. decipularis*, *D. stevensonii*, *D. tucurensis*, *D. spruceana*, *D. maritima*, and *D. cochinchinensis* were provided by the U.S. Fish & Wildlife Forensic Lab, all of which are listed as CITES Appendix II species, except for *D. nigra*, which is listed as a CITES Appendix I species. The headspace volatiles of the wood samples were concentrated on SPME fibers for thirty minutes and analyzed by DART®-HRMS. Multivariate statistical analysis processing of the DART®-HRMS data revealed intraspecies similarities and interspecies differences that resulted in the ability to assign species attributions to the chemical signatures. The classification model that was developed could therefore be used for forensic identification of species based on simple analysis of the headspace of the wood. The results show that this approach can be used as a technique for species identification of these illegally traded timber.

#### **Endangered Wood, DART®-HRMS, Illegally Traded Timber**

## **B188 Evaluation of Organic and Inorganic Gunshot Residues in Various Populations using LC-MS/MS**

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**Learning Overview:** After attending this presentation, attendees will understand the importance of utilizing IGSR and OGSR components and advanced algorithms for interpreting presented evidence.

**Impact Statement:** This presentation will impact the forensic science community by presenting findings of IGSR and OGSR occurrence from various subpopulations.

Differentiation and classification of trace materials are critical tasks in many criminal investigations yet still present a challenge for forensic science practitioners.<sup>1</sup> The formation of GSR is entropic in nature and yields varying and sometimes time-dependent analytes. GSR evidence is categorized into two constituents – inorganic particulates (IGSR) and organic compounds (OGSR). These analytes are formed after a firearm's firing pin strikes the primer of the ammunition, igniting the propellant and expelling a projectile at high velocities.

Two approaches evaluated the evidence for additional interpretation and understanding – classification via machine learning algorithms and estimation of the strength of the evidence via likelihood ratios. Neural network models can predict responses when exposed to large data sets through rigorous training measures such as backpropagation and cross-validation. Likelihood ratios are used to evaluate the evidence given two mutually exclusive hypotheses.

This work investigates the prevalence of organic and inorganic gunshot residue within two main subpopulations: (1) non-shooters, including groups with low- and high-risk of potentially containing GSR-like residues, and (2) individuals involved in a firing event (shooters, bystanders, and shooters performing post-shooting activities). The study analyzes over 400 samples from individuals via a recently validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology with complexing agents.<sup>2</sup>

Exploratory statistical tools and neural networks evaluated the quantitative data of the subpopulations. We report lower occurrences of OGSR compounds in the non-shooter (high- and low-risk) population compared to IGSR elemental profiles. By utilizing different strategies on the observed OGSR and IGSR traces, the presence of GSR from shooters from other potential sources, such as bystanders and some professions, can be further assessed. Additionally, the low-risk background sample set allowed documentation of GSR occurrence in the general population.

The probabilistic outputs of the neural network models were utilized to calculate likelihood ratios (LR) as a proxy for the significance of evidence. Through rigorous training of the model with combining IGSR and OGSR profiles, the false-positive rates obtained from the neural network were less than 5%. This evidence further suggests that this approach can be effectively used for a probabilistic interpretation of GSR evidence.

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

1. Gallidabino, M.D.; Barron, L.P.; Weyermann, C.; Romolo, F.S. Quantitative Profile- Profile Relationship (QPPR) Modelling: A Novel Machine Learning Approach to Predict and Associate Chemical Characteristics of Unspent Ammunition from Gunshot Residue (GSR). *Analyst* 2019, 144 (4), 1128–1139. <https://doi.org/10.1039/c8an01841c>.
2. Feeney, W.; Menking-Hoggatt, K.; Vander Pyl, C.; Ott, C.E.; Bell, S.; Arroyo, L.; Trejos, T. Detection of Organic and Inorganic Gunshot Residues from Hands Using Complexing Agents and LC-MS/MS. *Anal. Methods* 2021. <https://doi.org/10.1039/d1ay00778e>.

### **Gunshot Residue (GSR), Population Study, Likelihood Ratio**

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### **B189 The Association of Smokeless Powders and Lab-Generated Residues to Organic Gunshot Residue (OGSR) Using Gas Chromatography/Mass Spectrometry (GC/MS) and Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) Analysis Followed by Chemometrics**

*Emily Lennert, MS\*, University of Central Florida, Orlando, FL; Candice Bridge, PhD, University of Central Florida, Orlando, FL and National Center for Forensic Science, Orlando, FL*

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**Learning Overview:** After attending this presentation, attendees will understand how organic gunshot residues (OGSR) may be analyzed via direct analysis in real time – high resolution mass spectrometry (DART®-HRMS) and gas chromatography – mass spectrometry (GC-MS), and subsequently the application of chemometrics in determining the effectiveness of classification of OGSR within smokeless powder models.

**Impact Statement:** This presentation will impact the forensic science community by providing insight regarding how organic gunshot residue (OGSR) may be best analyzed via DART®-HRMS, as well as by traditional means of analysis using GC-MS, and how those residues may be associated with the intact smokeless powder (SP) particle. This study may provide a basis for potential evidentiary links between the residue recovered from a crime scene and a suspected SP from which the evidence may have originated.

Following a shooting, gunshot residues (GSR) settle on areas such as the shooter's hands and face, the shooter or victim's clothing, and other nearby surfaces. Organic gunshot residue (OGSR), which primarily originates from the smokeless powder (SP) contained in the ammunition, accounts for a portion of the GSR in addition to the inorganic GSR that is more commonly analyzed. Additionally, in explosive events such as the use of an improvised explosive device in which SP serves as the main charge, smokeless powder residues (SPR) may serve a similar purpose as OGSR as evidence.

In this study, SP samples were extracted following a modified version of the extraction protocol set forth by the National Center for Forensic Science.<sup>1</sup> Extracts were analyzed via GC-MS, a gold standard of analysis available in many operational crime labs, as well as DART®-HRMS, a newer technique. In both analyses, key organic components of the SP were easily identified in the extracted material. Subsequently, classification models were generated from a series of chemometric analyses. Then, SPRs were generated in-lab through a small-scale burning method in which SP samples were individually ignited within foil packets and extracted similarly to the intact SP. Resulting data from each instrument was classified within the respective SP model using linear discriminant analysis with the SPR as an external test set. Results indicated generally low classification accuracy, and thus SPR models were also generated.

Finally, 9 mm ammunition was loaded with known SP and fired to collect OGSR from cloth targets and cartridge casings. The OGSR was extracted and analyzed via DART®-HRMS and GC-MS and tested against the SP and SPR models to determine the association of OGSR to its intact SP to allow for narrowing the source of evidence, as well as lab generated SPR for the assessment of the representativeness of the burn method. The ability to characterize a SP, SPR, or OGSR and successfully associate these to one another may be helpful in determining the origin of a suspected sample and aid investigators in linking a suspect to a crime scene.

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

- <sup>1</sup> Smokeless Powders Database [internet]. National Center for Forensic Science, University of Central Florida. Available from: <http://www.ilrc.ucf.edu/powders/>.

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#### **Organic Gunshot Residue, GC/MS, DART®-HRMS**

## **B190 Elemental Profiling of Total Gunshot Residue (tGSR) Using Total Reflection X-Ray Fluorescence (TXRF) Spectrometry**

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**Learning Overview:** The aim of this presentation is to help attendees understand the utility of total reflection X-ray fluorescence spectroscopy in producing elemental profiles of total gunshot residue produced from the firing of different ammunition.

**Impact Statement:** This presentation will present a novel approach to qualitative and quantitative analysis of total gunshot residue found on cloth, and in differentiating various ammunitions based on their elemental profiles.

The aim of this presentation is to help attendees understand the utility of total reflection X-ray fluorescence spectroscopy in producing elemental profiles of total gunshot residue produced from the firing of different ammunitions.

This presentation will present a novel approach to qualitative and quantitative analysis of total gunshot residue found on cloth, and in differentiating various ammunitions based on their elemental profiles.

Gunshot residue (GSR) is composed of combustion products from the various components of firearm ammunition cartridges. These components include propellant powder, primer, grease, lubricants, and the different metals that make up the bullet.<sup>1</sup> Traditionally blasting particles are the main type of investigated GSRs, most commonly found on the shooter hands. This type of GSR can present as whole particles, flattened particles, or partially splattered, which is usually collected by swabbing or tape-lifting and analyzed by a scanning electron microscope and energy dispersive X-ray spectroscopy (SEM-EDS).<sup>1,2</sup> SEM-EDS, while simultaneously imaging and identifying GSR particles, only analyzes approximately one square millimeter worth of GSR and does not analyze other types of GSR such as impact deposition residue.<sup>3</sup> Other studies have used instrumentation such as inductively coupled plasma mass spectrometry (ICP-MS) to quantify GSR deposited on surfaces such as wood and metal, but the methodology only involved swabbing the interior surface of the bullet hole. total reflection X-ray fluorescence spectrometry (TXRF) is a highly sensitive qualitative and quantitative method that for elemental analysis.<sup>3,4</sup> Absolute quantities can be determined with a spiked internal standard such as gallium or yttrium. This instrumental technique employs a molybdenum or tungsten X-ray source to eject an inner shell electron before an outer shell electron fills the resultant void and emits X-ray fluorescence with energy that is characteristic to elemental identity. The X-ray fluorescence intensity or photon counts can be used for quantitation. In contrast to SEM-EDS, TXRF can quantify a greater number of elements in each run with lower energy consumption. Different from ICP-MS, TXRF is a type of elemental analysis often free from complex ion interference, without bulky and noisy vacuum pumps. In this study, TXRF was used to study total gunshot residue (tGSR), the collection of all deposits on a target. Six types of ammunition were fired at targets of plain cotton using two firearms from a fixed distance of six inches. The samples were individually digested in concentrated nitric acid for a minimum of 24 hours to dissolve the GSR particles prior to analysis.

Through TXRF analysis, different types of ammunition were found to produce different and unique elemental profiles of tGSR. The focus of the elemental analysis was on lead, copper, barium, antimony, iron, and zinc. Other detected elements were excluded as they were found in similar quantities between the environmental blank and the samples. Lead, as the main element composing the bullet, was the primary element of interest and was found to corresponds to the amount of a traditional bullet that is exposed to the target and the combustion heat upon discharge of the firearm. For example, the unjacketed bullet had a lead concentration of  $239.25 \pm 1.32$  ppm while the full covered total metal jacket ammunition had a concentration of  $4.56 \pm 0.02$  ppm. The second element of interest was copper, which corresponded to the composition of the bullet and the amount of jacketing on the different ammunitions. For example, the unjacketed ammunition had the lowest concentration of copper at  $0.23 \pm 0.44$  ppm, as expected. The TXRF analysis produced repeatable and reliable elemental profiles in which the information could be used as the basis as a predictive tool to associate ammunition types and the impact deposition on a close-range target.

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### **Gunshot Residue (GSR), Total Reflection X-Ray Fluorescence Spectrometry, Elemental Profiling**

## B191 A Pilot Study of Lead Isotopes and Trace Element Utility in Shooting Investigations

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**Learning Overview:** After attending this presentation, attendees will understand the potential for chemically associating bullet fragments and impact marks with specific bullets by ICP-MS and MC-ICP-MS for shooting investigations.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new method to associate bullet impact marks identified with sodium rhodizonate lifts with a specific bullet from a limited pool of possibilities.

Previously, the FBI testified that a bullet could be determined from a specific box of bullets. Due to manufacturing and packaging practices, testimony using this technique was not scientifically supported according to the National Research Council and has been discontinued.<sup>1-3</sup> However, inferences from lead isotopes combined with trace elements in bullets may still prove useful in forensic shooting investigations.<sup>4-6</sup>

Bullet manufacturers use inexpensive sources of lead, which vary with time, shipping costs and the development cycle of lead mines.<sup>2</sup> During packaging, bullets in a specific box may come from multiple lots of lead. Hence, associating a single bullet with a specific box of bullets is highly problematic. However, it is demonstrated here that single bullets are relatively homogenous in both element ratios and lead isotope compositions, and residue from the bullet can be probabilistically associated with the main bullet fragment. The hypothesis was, given a shooting incident with a limited number of projectiles (a “limited universe”), most of the projectiles can be eliminated from consideration as having made a specific ricochet mark or left a specific fragment in a wound track.<sup>7</sup>

Six bullets and five evidence lifts made from ricochet marks were anonymized and sent by Luke Haag of Forensic Science Services to researchers at Arizona State University. Six bullets were provided, ensuring associations between lifts and bullets were not made by process of elimination. Bullets were swabbed using dilute nitric acid, minimizing damage to the evidence.<sup>7</sup> A method to solubilize the lead from evidence lifts was developed, and an optimized method for measuring low concentrations of trace elements (Cu, As, Ag, Sn, Sb, Ba, Bi) in the presence of very high lead concentrations using ICP-MS. This method optimization included use of (1) a custom in-house internal standard (Sc, Ge, In); (2) developing a matrix-matched calibration curve to quantify very low trace elements despite interferences from the high lead matrix; (3) maintaining constant lead concentrations for samples ensuring high quality trace element data close to the limit of quantitation; and (4) optimization of sample concentration to avoid impact of high lead samples on substantial lower concentration lead samples. This method uses element ratios, rather than absolute concentrations of trace elements, because the initial mass of bullet lead recovered by solubilizing material from an evidence lift cannot be determined. Samples were then diluted and measured for lead isotopes by MC-ICP-MS. Lead isotopes were superior to trace elements to accurately discriminate between similar possible lift-bullet pairs. Associations between the blinded lifts and bullet pairs made by the researchers were confirmed to be 100% accurate to the known associations by Luke Haag.

Overall, this pilot study concluded this new method was reliable and effective at correctly associating evidence lifts with bullets, using elemental/isotopic ratios and isotopic plots. This research is continuing in collaboration with Luke Haag and Chris Gunsolley, a Forensic Scientist Firearms Supervisor at Mesa Police Department. In a series of shooting experiments, the accuracy and specificity of this method for associating GSR from cotton shirts, bullet fragments recovered from ballistic soap, and bullets from a single shooting event is being evaluated. If successful, this technique could be widely used in shooting investigations.

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### Shooting Investigations, Bullets, Lead Isotopes

## **B192 A Study on the Longevity of pGSR in an Outdoor Environment.**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how long pGSR will last in an outdoor environment.

**Impact Statement:** This presentation will impact the forensic science community by providing practitioners insight into whether pGSR remains in an outdoor environment after approximately 13 years. The presentation also describes the methods used to test this theory.

Gunshot primer residue (GSR) is produced by a firearm when it is discharged. The primer for centerfire cartridges is mainly composed of lead styphnate, barium nitrate and antimony sulfide. The residue from the primer explosion escapes from openings in the gun and is deposited on nearby surfaces including a person's hands and clothing. These particles can be collected and analyzed using automated scanning electron microscopy energy dispersive x-ray spectrometry (SEM/EDS). Characteristic gunshot residue primer particles have a molten appearance and are composed of barium, antimony, and lead.

There have been numerous studies addressing the longevity of pGSR on hands and clothing. There have not been any studies on whether pGSR can be kicked up from the surface of the ground onto a person's clothing. The laboratory was fortunate enough to be able to access the department's old outdoor firing range which had not been in service since 2008. GSR stubs were taken not only of some of the surfaces but an analyst wearing clean clothes, walked and kicked up the ground in the range. Stubs were taken from multiple areas of his shoes, clothing and hands. These stubs were analyzed using scanning electron microscopy energy dispersing x-ray spectroscopy instrumentation using standard laboratory procedures for the analysis of gunshot primer residue.

The presentation will detail the results of a study on gunshot primer residue found in an outdoor environment transferring to an individual walking through that environment. This research takes a novel approach by investigating the likelihood of transfers from the ground to other surfaces including hands.

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### **Gunshot Residue (GSR), Longevity, SEM**



## **B193 Probabilistic Interpretation of a Large Population Study of Gunshot Residue and Background Profiles Using LIBS, Electrochemistry, and SEM-EDS**

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**Learning Overview:** After attending this presentation, attendees will understand the strength of orthogonal methods for identification of gunshot residue (GSR) and improved classification and evidential value when using probabilistic interpretation.

**Impact Statement:** The findings on IGSR and OGSR in various populations and the use of probabilistic interpretation are anticipated to provide a significant leap in knowledge in the field of gunshot residue.

This study aims to address some research needs identified in the field of GSR, such as studying the prevalence of OGSR and IGSR in the general population and evaluating the feasibility of using organic and inorganic data for more comprehensive analysis and interpretation approaches.

To address these gaps, we conducted a large population study of over 3,000 samples originating from various sources of known shooters' hands ("shooter") and background populations ("non-shooter"). A large study of both "shooter" and "non-shooter" samples assists with understanding the trends of elemental combinations present in authentic specimens, along with environmental residues that could be misidentified as GSR.

Five subpopulations were studied. The known shooter samples originated from firing pistols and revolvers using various ammunition types: leaded (set 1), lead-free (set 2), and a mixture of both (set 3). Background samples were collected from individuals who had not fired a gun in the past 24-hours, separated by low-risk (set 4) and high-risk (set 5). Low-risk samples were collected from volunteers who did not have hobbies or professions that could mimic GSR, while high-risk included firearm researchers, agriculture workers, mechanics, and police officers/administrators. Samples were first analyzed by LIBS, which can detect multiple elements of interest and provides 25 spectra in less than 2 minutes with spatial information. Then electrochemistry was completed on the same sample, simultaneously detecting both IGSR and OGSR (Pb, Cu, Sb, 2,4-DNT, DPA, nitroglycerin, and ethyl centralite) in under 3 minutes. Lastly, a subset from each population was analyzed by SEM-EDS for morphological and elemental information, demonstrating SEM-EDS could confirm GSR after screening with the novel methods. The sole presence of Pb was found in the low-risk, high-risk, and leaded shooter populations at occurrences of 26.8%, 64%, and 83%, respectively. Barium is highly prevalent (94% in low risk, 100% high risk, 100% in shooters). Conversely, some GSR elemental combinations, such as PbBaSb were present in 100% of the leaded shooter samples, in 16% of the high-risk, and only 1.8% in the low-risk background. The presence of lead-free elements was more frequent in background sets (up to 64% TiZn or 92% Cu). OGSR compounds such as NG were detected in shooter sets while remained absent in the background populations. Finally, combinations of OGSR and IGSR were absent in background sets, indicating enhanced certainty when observed in GSR.

The data was classified using a categorical exploratory method (critical threshold) to estimate positive and negative rates, and the performance was compared to machine learning algorithms. Regardless of the classification method, accuracy of 87% or higher was observed for individual techniques and 92% or higher when combined (LIBS and EC). The machine learning probabilistic outputs were used to calculate log<sub>10</sub> likelihood ratios which were typically between -2.5 to -5 for non-shooters and >5 for shooters, demonstrating good discrimination between the overall populations and the viability of using LR as a proxy for reporting the weight of the evidence. Tippet plots were used evaluate LR and the rates of misleading evidence (RME <3.7%).

The probabilistic interpretation and use of likelihood ratios provide additional support to examiners and the trier of fact to better inform their decisions about the weight of the evidence. The incorporation of fast emerging methods, and the information derived from this large population study of IGSR and OGSR, combined with probabilistic interpretation, will contribute strengthening GSR evidence.

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### **Analytical Methods, Gunshot Residue (GSR), Probabilistic Interpretation**

## B194 Acid Phosphatase Detection in Food Products and Mold

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**Learning Overview:** After attending this presentation, attendees will understand that many food items and fungi, including mold, contain detectable levels of endogenous acid phosphatase (AP) and will yield positive results using the forensic AP Spot test.

**Impact Statement:** This presentation will impact the forensic community by demonstrating that the AP Spot test for semen can yield positive results in the presence of certain foods and fungi, even though semen is absent.

Semen is frequently encountered on sexual assault evidence and is often detected using a colorimetric test for acid phosphatase (AP). Other substances that are known to contain detectable levels of AP include vaginal secretions, saliva, urine, certain foods, plants, and other miscellaneous material including fungi. Fungi are spore-producing, eukaryotic organisms that include a wide variety of species such as molds, yeasts, and mushrooms. Medications, illicit drugs, bread, alcohol, fermented foods, food colorants, vitamins, preservatives, and pesticides are derived from fungi, and may come into contact with or contaminate an object of forensic relevance. Food and other items are susceptible to fungal growth when left in improper conditions, such as damp spaces and warm temperatures. Fungi also grow unwanted in human spaces, notably in the form of mold in damp, enclosed areas. Thus, it is possible that evidentiary items exhibiting mold growth or those that have been in contact with mold or food (such as items recovered from a trash receptacle) could test positive for the presence of AP, even in the absence of semen.

This study had three primary goals: (1) to determine whether known cultured molds and unknown home-grown molds would produce positive results with an AP spot test; (2) to determine whether the presence of mold on non-semen biological stains would produce positive results with an AP spot test; and (3) to determine whether any AP-positive mold samples would produce positive p30 or Semenogelin (Sg) results in the absence of semen.

Numerous non-moldy food items, moldy food items, cultured mold spores, and semen-free body fluid stains were obtained and deposited onto individual filter papers or tested directly using AP Spot reagent (SERI, Richmond CA). Of 26 non-moldy food and yeast samples tested, 16 showed a positive violet color change result within the 5-minute observation period; 4 of those items (butter bread, banana, pear and cremini mushroom) showed a strong positive reaction within 1 minute. Following mold growth on 25 food items, AP-positive results were obtained for six yeast-based bread products, five vegetables, three unknown foods from a shared refrigerator, one dairy product, and one fruit. Further, a tree fungus specimen and 13 of 15 mold culture samples had detectable levels of AP, many with a reaction time under 2 minutes.

A total of 17 fresh or frozen urine, saliva, and vaginal fluid samples were tested for AP. Two saliva samples showed a strong positive result and were excluded from further study. Of the remaining samples, 1 vaginal sample was negative, and 14 samples gave a faint positive reaction occurring at the 1-minute mark or later. All samples but one subsequently developed visible mold spores over a 2-month period. Following mold growth on these semen-free samples, all gave a positive reaction for AP, with two urine samples, one saliva sample, and one vaginal fluid sample showing a more intense color change than before mold growth, with some developing as quickly as 30 seconds. A total of 27 AP-positive samples were tested using both ABACard® p30 immunoassay cards and RSID™-Semen immunoassay cards. All p30 and Sg testing yielded negative results.

This study demonstrates that a variety of foods, yeast and mold have detectable levels of acid phosphatase using the AP Spot test; however, semen-free moldy samples with detectable levels of AP did not yield positive p30 or Sg testing results. Thus, evidence that has developed mold or comes into contact with mold spores or certain food products should be interpreted with caution when positive AP Spot test results are obtained, particularly when subsequent protein testing and microscopic analysis for sperm is negative.

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### Acid Phosphatase, Mold, AP Spot Test

**B195 Reducing Interference in the Ultraviolet/Visible (UV-Vis) Spectra of Blood Samples to Detect Ethylenediaminetetraacetic Acid (EDTA) Via the Reverse EDTA Detection Using Eriochrome® Black T (EBT) and UV/Vis (RED-BLEU) Assay**

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**Learning Overview:** After attending this presentation, attendees will understand how to reduce interference from blood proteins in a UV-Vis spectrum as part of the RED-BLEU (Reverse EDTA Detection in Blood using EBT and UV-Vis) assay.

**Impact Statement:** This presentation will impact the forensic science community by contributing to the authenticity of blood evidence via a presumptive test for EDTA.

Assertions of false blood evidence are not unheard of in the courtroom. Thus, developing a rapid method to refute or support these claims would contribute to increased transparency in criminal cases. When blood is drawn as a reference sample, it is typically preserved with the anticoagulant ethylenediaminetetraacetic acid (EDTA). EDTA is not found naturally in blood; therefore, its presence in an evidentiary sample could indicate planted blood.

A colorimetric test for the presence of EDTA in blood using Eriochrome® Black T (EBT) indicator (Reverse EDTA Detection or RED) has been developed. To reduce false positives and enhance the robustness of this presumptive test, it was coupled with ultraviolet-visible spectroscopy (UV-Vis) immediately following the colorimetric reaction, and has therefore been collectively termed the RED-BLEU assay.<sup>1,2</sup> While the use of the NanoDrop™ 2000 spectrophotometer also enabled quantification of EDTA within samples, other components within blood exhibited substantial interference with this portion of the assay.<sup>1,2</sup>

The primary goal of this research was to develop a treatment for blood samples to reduce interference in the UV-Vis spectrum to the point that detection of EDTA is possible. Several efforts were attempted to reduce this interference, including protein precipitation and protein denaturation. Protein precipitation was attempted using ammonium sulfate and trichloroacetic acid (TCA), both of which are used in blood sample preparation for other analytical techniques.<sup>3,4</sup> Proteinase K was also explored in an attempt to denature the interfering blood proteins. To evaluate these, mock samples were created with HPLC-grade water, calcium and magnesium ions, and EDTA (positive samples only). Blood samples with and without EDTA were also used for testing each method. Blood samples were treated both before and after processing with the EBT indicator test to identify any interference(s) with the color changes, as well as to observe the impact of the colorimetric reagents on the precipitation method.

When used prior to the RED assay, ammonium sulfate produced a blue color (positive result) in all samples, regardless of the presence/absence of EDTA, and did not yield an observable reduction in the amount of inference from blood in the UV-Vis spectra. When a TCA treatment was applied prior to the RED test, the expected results were achieved for the colorimetric portion of the assay; however, this treatment also did not afford observable changes in the UV-Vis spectra. Although a Proteinase K treatment applied prior to the RED test interfered with that portion of the assay, this treatment did reduce interference from blood components in the UV-Vis spectra. Therefore, a Proteinase K treatment between the colorimetric and UV-Vis portions was evaluated and demonstrated the most promise for removing the blood protein interference without compromising the colorimetric test. Additional studies are needed to optimize this Proteinase K treatment for integration into the RED-BLEU assay.

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**RED-BLEU, Blood, Ethylenediaminetetraacetic Acid (EDTA)**

## B196 Volatile Organic Compounds (VOCs) Associated With Diseased Human Blood

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**Learning Objective:** Those attending this presentation will have a better understanding of the volatile organic compound (VOC) profile throughout decomposition and how health conditions of the deceased can affect the profile.

**Impact Statement:** Characterizing the differences between the volatile organic compounds (VOCs) produced by healthy and diseased blood may enhance our understanding of the body's health-state on decompositional processes.

According to the CDC, 6 in 10 adults are living with a chronic disease.<sup>1</sup> Blood serves as a repository of information regarding one's health state. One means of datamining this "blood bank" is to identify the VOCs that are present in fresh blood as well as those produced by decomposing blood.<sup>2</sup> Indeed, disease-specific blood VOCs are of interest in clinical settings for rapid disease diagnosis and may provide insight on cadaveric state.<sup>3,4</sup> A number of factors influence VOC composition of decomposing blood such as temperature, moisture, soil composition, and health of the individual among other factors.<sup>2,5</sup> Each of these factors influence the post-mortem interval (PMI) estimation by forensic pathologists and may interfere with search efforts by human cadaver dogs.<sup>2</sup> Previous research has focused on environmental factors, drug use, and blood alcohol levels, but there is little information regarding diseased human blood VOC composition.<sup>6</sup>

Headspace solid-phase microextraction (SPME) paired with gas chromatography-mass spectrometry (GC-MS) was used for the detection and characterization of the volatile components of decomposing blood samples. Nondiseased, anemic, and diabetic blood samples were placed in uncapped glass vials and allowed to stand for two weeks. VOCs were collected at 0, 24, 96, 168, and 336 hours. Thirty minutes prior to collection, the vials were capped with pressure-tight teflon caps to trap released gases. An SPME fiber was then inserted into a vial's headspace for sample adsorption. After fiber removal, the vials were then left uncapped until the next sample cycle. The SPME fiber was then sampled using the GC-MS to identify the volatile organic compounds. Throughout this research, diseased and nondiseased blood VOC profiles consistently differed. Overall, nondiseased blood produces more abundant and more varied VOC emissions compared to anemic and diabetic human blood.

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### Decomposition, Volatile Organic Compounds (VOCs), Blood

## B197 Stages of Heat Damage to Fabrics Encountered in Forensic Clothing Examination: Microscopic Examination

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**Learning Overview:** Attendees will gain awareness that microscopically observable evidence of types and degrees of heat damage to fabrics provides information that can be correlated with conditions at a scene or exposure of a wearer to a heat source.

**Impact Statement:** Findings of different stages of thermal damage to fabrics can provide evidence of exposure to different temperatures and degrees of heat, such as from polymer softening due to heat generated upon impact friction, from exposure to flame, contact with a hot surface, or from sticky residues from cellulose pyrolysis of nearby materials.<sup>1</sup> The types and degrees of damage can be useful in answering case questions.

Effects of heat exposure can be found on clothing items as either thermal alteration to the clothing itself, or deposits of other heat-altered materials. The latter may occur when fabrics are damaged together or were removed and burned together to destroy evidence. In this study, fabrics comprising a single type of fiber were exposed to flame and conduction heating both singly and paired with another fabric.<sup>2</sup> Transfer demonstrated in prior heat studies using cotton blue jeans and t-shirts includes ash/char fragments and amber residues produced as aerosols during cellulose combustion; the droplets can drift to a person standing nearby.<sup>3</sup>

To provide a basis for correlating observed damage with the conditions that produced it, the stages of heat damage observable with a stereomicroscope (6x to 40x magnifications) were described, classified and photographed. Follow-up examinations of fabric fragments and constituent fibers were conducted using polarized light microscopy (PLM).

For description of stages of damage, fabric and fiber types were divided into three categories: cellulosic, i.e., plant fibers and regenerated cellulose; animal (protein-based) fibers; and synthetics. Stages of heat damage in cellulosic fibers include scorching, charring; and pyrolysis with thermal decomposition as described earlier. Damage to proteinous fibers such as wool and silk is characterized by discoloration followed by melting and thermal decomposition; bubbles produced by gasses during pyrolysis are observed in individual fibers; and finally, hard dark crusts are formed. Loss of birefringence is observed. In wool, as in other hair, initial swelling results from expansion of air spaces. Most synthetic fibers soften, melt, then undergo thermal decomposition with hardening. Acrylics typically decompose before melting. All exhibit loss of birefringence upon softening in the polymer transition temperature region.

A match held to the side of each fabric test swatch produced a temperature gradient from edge to interior; other swatches were heated uniformly in tins on a hot plate. Selected fabrics were wrapped in aluminum foil and heated with a butane utility lighter to produce higher temperatures. Isolated areas of each fiber type are more readily observed at higher magnifications (100x, 250x and 400x). Preliminary tests of blended fabrics, comprising more than one type of fiber, also suggest that areas individual fiber types can be found. Both these topics will be the focus of future studies.

Correlations of visually observed stages of heat damage with underlying chemical changes, studied via FTIR, are reported in a companion study.

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3. Shafizadeh, Fred. The chemistry of pyrolysis and combustion. In: Rowell, Roger. *The chemistry of solid wood*, Vol. 207, Chapter 13, pp 489-529. American Chemical Society: May 1984.

### Thermal Damage, Forensic Clothing Examination, Fibers

## B198 The Effect of Washing on the Transfer and Persistence of Fiber Evidence

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**Learning Overview:** After attending this presentation, attendees will gain an understanding of (1) primary, secondary, and tertiary transfer of fiber evidence via washing and drying; and (2) persistence of fibers on clothing after washing and drying.

**Impact Statement:** This presentation will impact the forensic science community by providing insight as to how washing affects the transfer and persistence of the two most common fiber types, cotton and polyester, which can be used to inform the evidentiary significance of fiber evidence.

Fiber evidence has proven to be valuable forensic evidence in a plethora of cases by providing associations between a suspect, victim, and/or location. This can most readily be seen in the Wayne Williams murder trial of 1984, where unique fibers served as linkages between victims and the suspect's home.<sup>1</sup> Specific characteristics of fibers such as shedability, cross-sectional shape, colorant (dye or pigment), and fiber type (natural versus synthetic) can be observed in order to determine the rarity of the fiber, which can be used to assess the evidentiary significance of an association.<sup>2,3</sup>

A gap in the literature has been identified regarding the effect of different variables, specifically washing, on the transfer and persistence of fibers. This research has been based on the underlying assumption that a perpetrator will wash clothing that was worn during the crime in an attempt to remove any evidence. Four different scenarios were displayed in this research, with target fibers on the donor garments being fluorescently dyed for means of easy identification. These scenarios included washing a single donor garment, washing the donor garment with a recipient garment, washing the donor garment with two recipient garments, and finally washing a single donor garment, taking it out, and washing a single recipient garment. These sets of washes were run for both cotton and polyester donor fibers. After washings were complete, the individual garments were bagged, labeled, and stored for examination using fluorescent photography.

Whether it be a primary, secondary, or tertiary transfer, after examination and documentation, fibers were observed to have been transferred in all scenarios from donor to recipient garments as well as secondary locations such as the inside of the washer and/or dryer. There were also major differences noted in the quantity, location, and size of the recovered fibers based on the number of garments washed as well as the donor fiber type. By understanding how washing affects transfer and persistence of different fibers, forensic scientists can be better informed of the recovery potential and location, as well as evidentiary significance of fibers from washed garments.

### Reference(s):

1. Deadman, H.A. (1984). *Fiber Evidence and the Wayne Williams Trial* (pp. 1-11) (United States, Federal enforcement Bulletin.)
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### Fibers, Transfer, Persistence

## **B199 The Identification of Toxic Adulterants in Seized Drug Material Around the World**

*Judith Rodriguez Salas MSFS\**, *The Center for Forensic Science Research and Education, Willow Grove, PA*; *Amanda Mohr, MSFS, The Center for Forensic Science Research and Education, Willow Grove, PA*; *Thom Browne, MA, Colombo Plan, Colombo, Sri Lanka*; *Barry Logan, PhD, The Center for Forensic Science Research and Education, Willow Grove, PA and NMS Labs, Horsham, PA*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of identifying toxic adulterants in seized drug material.

**Impact Statement:** This presentation will impact the forensic science community by bringing awareness of the potential health risks that adulterants can create and how is important to report them.

The International Adulterant Database (ITAD) was created in 2018 as a result of the first Symposium of Forensic Drug Testing Lab Directors held in December 2017, where 33 laboratories from 22 different countries attended. The creation of the database resulted from discussions related to problems with toxic adulterants and diluents found in seized drug materials, and the public health risks associated with their use. Toxic adulterants, pharmacologically active substances and diluents, pharmacologically inactive substances, are regularly used as cutting agents and intentionally added to illicit drugs. Toxic adulterants are of concern because potential increased toxicity related to their actions on the central nervous system.

For the past four years, laboratories from all over the world have collected and sent in data that they gathered from their analysis of seized drug samples. Submitted data included information on illicit drugs, toxic adulterants and diluents identified within seized material. To date, 16 different countries have submitted data to the ITAD database. Countries provided data either monthly, quarterly, yearly or biannually. The data was collected and analyzed using Microsoft Excel®.

The toxic adulterants reported to ITAD can vary between countries and the illicit drug type, but many adulterants are reported with some frequency. Seized cocaine samples have been reported to contain caffeine, levamisole, lidocaine, phenacetin and benzocaine, alone, and in a in various combinations of these substances. Toxic adulterants identified in seized heroin samples included caffeine, acetaminophen, dextromethorphan, lidocaine, phenacetin and diltiazem, along with other by-products related to illicit manufacturing process. MDMA pills have been identified mostly with caffeine, levamisole and acetaminophen.

Looking specifically at individual countries, in Argentina, 46% of cocaine samples were unadulterated, 36% contained caffeine, 24% lidocaine and 5% phenacetin. In Brazil, all cocaine samples were identified with an adulterant. The most commonly encountered one was phenacetin in 48%, followed by levamisole (31%), and hydroxyzine (22%). With respect to heroin samples, samples from Saudi Arabia were adulterated with caffeine (36%), dextromethorphan (26%) and acetaminophen (8%). Comparatively in Singapore, 92% heroin samples were found with caffeine, followed by chloroquine in 78% and acetaminophen in 45%.

Most of the time, these toxic adulterants do not pose significant health risks when ingested in small amounts or used as prescribed. However, the unknown concentration of these adulterants and the mixture of one or more with the illicit drugs and chronic use can results insignificant. Caffeine, at small doses is innocuous but at toxic levels it can create convulsions and cardiac arrhythmias. Levamisole, a drug approved by the FDA in 1990 for the treatment of cancer, was withdrawn of the market a decade later due to the severe side effects such as, agranulocytosis (lowered white blood cell count) and skin necrosis (skin death). These are some of the most severe side effects of this drug.

The identification of toxic adulterants is important because there are risks associated with unknowingly ingesting these compounds. Based on the data available in the ITAD database, a wide variety of adulterants and combinations are commonly detected in seized material across several countries for the same illicit substance. Further, adulterants likely to be detected are largely dependent on the country of origin and countries that may be close in proximity can have differences in the adulterants that are used. It is important to bring awareness related the different adulterants that can co-ingested with the illicit substances as they can contribute to adverse effects that may lead to health complications for the users or even death.

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### **Toxic Adulterants, Seized Drugs, Health Risks**

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## B200 X-Ray Powder Diffraction: A Unique Approach for Identifying and Differentiating Controlled Substances

*Hillary Culbertson, PhD\*, Defense Forensic Science Center, Forest Park, GA*

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**Learning Overview:** After attending this presentation, attendees will have an understanding of XRPD analysis of controlled substances as well as its benefits and drawbacks.

**Impact Statement:** This presentation will inform the forensic community about how x-ray powder diffraction (XRPD) can identify and differentiate controlled substances, how to create a high-quality XRPD library of controlled substances and cutting agents, and how an XRPD library of controlled substances is beneficial to a forensic lab.

X-Ray Powder Diffraction (XRPD) is non-destructive and yields highly discriminatory data characterizing the crystalline structure based on a chemical's composition. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) categorizes XRD as a Category A technique for the analysis of controlled substances based on its level of selectivity. Additionally, XRPD is rapid, compatible with mixtures, and requires minimal sample preparation. However, despite all of these analytical advantages, its use for the identification of controlled substances in the forensic drug chemistry field has infrequently been applied historically. The aim of this work was to assess the discriminating power of XRPD and its ability to identify controlled substances, and, if suitable, create an internal spectral library.

Proof of concept and method robustness were first established using codeine (sulfate), amphetamine (sulfate), methamphetamine (HCl), and cocaine (sulfate). Amobarbital (Na) and secobarbital (Na) were analyzed to demonstrate that controlled substances of similar chemical structures could be differentiated by XRPD. Additional analyses demonstrated that co-drugs and their constituents, positional isomers, homologs, and diastereomers could be differentiated by XRPD. XRPD was able to differentiate absolute enantiomers, but only when they had different salt forms.

Follow-on work involved creating a high-quality spectral library of controlled substances and common cutting agents. High-quality diffractograms of forty-three pure standards were acquired over the course of three hours. Each standard was analyzed three times, with the standard being removed and reapplied to the same zero background holder each time to ensure sample preparation did not influence the diffractograms. The resulting library has allowed drug chemistry examiners to detect and differentiate commonly encountered controlled substances and cutting agents in pure form or mixtures without sample degradation or consumption.

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### Controlled Substances, XRPD, Library



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## **B201 Evaluation of Two Portable Devices for the Detection of Common DFC Drugs**

*Hannan Latif, MSc\*, Queen Mary University of London, London, England, United Kingdom; Nikolas Petros Lemos, PhD, Queen Mary University of London, London, England, United Kingdom*

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**Learning Overview:** In attending this presentation, the attendee can expect to learn about drug facilitated crimes, particularly drug facilitated sexual assaults, and about two currently available portable devices that could potentially be used to decrease the incidence of such crimes. After attending this presentation, attendees will be able to appreciate the fact that the devices that claim to be able to detect drugs in drinks need to be further configured for this purpose before routine use in forensic settings can be allowed.

**Impact Statement:** This presentation will impact the forensic community by introducing the idea of using portable devices to detect drugs mixed in drinks, which are characteristic of crimes such as drug facilitated sexual assaults. By evaluating currently available devices, and configuring them for this purpose, they could be used in forensic fieldwork settings in cases where drugs have been used to facilitate the crime.

Drug Facilitated Crimes (DFC) such as Drug Facilitated Sexual Assaults (DFSA) are a growing public health concern with presumed underrepresentation due to underreporting. Several testing devices for the detection of drugs typically encountered in DFSA are commercially available to the public but need to be evaluated for their effectiveness so that there is no cause for concern regarding their use, degree of accuracy, result falsehood, and other factors encountered in forensic work.

Our study evaluates one such device consisting of a handheld NIR spectrophotometer. SCiO<sup>®</sup>, developed by Consumer Physics<sup>®</sup>, is a handheld pocket-sized device operating in the NIR. This device was used to scan drinks before and after the addition of a drug. 12 different drugs were tested in 12 different drinks. The drink without the addition of the drug was used as the negative control. Additionally, CYD<sup>®</sup> paper test strips were used to screen for the presence of ketamine and GHB in 10 of the 12 drinks.

Average NIR spectra were generated using the SCiO<sup>®</sup> handheld device and were evaluated using the SCiO<sup>®</sup> Lab application available of the world wide web. 6.3% (9/144) of the samples had a noticeable spectral shape difference only when compared to the negative control, 10.4% (15/144) had a noticeable intensity difference only when compared with the negative control and 22.2% (32/144) had both a noticeable spectral shape as well as intensity difference from the negative control. Collectively, 38.9% (54/144) of the samples had at least one noticeable difference between the spectra generated with and without the addition of drugs within the drinks. CYD<sup>®</sup> strips gave negative results for the presence of GHB and ketamine in all 10 of the drinks tested for.

Our study results suggest that although there was low efficiency of this device in detecting drugs within the drinks, SCiO<sup>®</sup> could potentially be optimized to be used for screening of drugs, particularly in drinks with low alcohol content. Additionally, SCiO<sup>®</sup> was deemed better able to detect drugs found in higher concentrations within the drinks but this scenario is often incompatible with the modus operandi of DFC perpetrators who aim to achieve the biggest effect with the smallest dose administered.

In summary, neither CYD<sup>®</sup> nor SCiO<sup>®</sup> are effective in the fight against DFCs in their current configurations and we propose that further research be conducted before these devices are marketed to the public for DFC detection/prevention.

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**Drug-Facilitated Crimes (DFC), Drug-Facilitated Sexual Assault (DFSA), Portable NIR Spectrometer**

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## **B202 Understanding Research Methods, Limitations, and Appropriate Applications of Drug Data Collected by the National Forensic Laboratory Information System (NFLIS-Drug)**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the limitations and appropriate applications of NFLIS-Drug data.

**Impact Statement:** This presentation will impact the forensic science community by presenting the most common pitfalls associated with using NFLIS-Drug data, including the reasons why these issues are problematic from a methodological perspective.

The National Forensic Laboratory Information System (NFLIS) is a drug surveillance program of the U.S. Drug Enforcement Administration that systematically collects data on drugs that are seized by law enforcement and submitted to and analyzed by the Nation's forensic laboratories (NFLIS-Drug). The NFLIS-Drug component represents all 50 U.S. State systems as well as scores of local and municipal laboratory systems that cumulatively account for more than 98% of the national drug caseloads among forensic laboratories with drug chemistry sections.

This presentation provides an overview of NFLIS-Drug data, which are increasingly being used in predictive modeling and to examine drug use patterns. Based on analysis of 428 unique, refereed article citations published between January 2005 and April 2021, eight concerns that have important implications for proper interpretation and limitations of NFLIS-Drug data were identified. The concerns pinpointed are 1) NFLIS is a voluntary program with varied reporting coverage. Users of NFLIS-Drug data obtained through request outside of NFLIS reports are not privy to the details of partial or interrupted reporting or other coverage issues. 2) Not all drugs are tested by crime laboratories. 3) NFLIS-Drug counts reflect "drug reports." Not all drugs that are seized or submitted for testing are assessed. 4) There are differences in laboratory reporting and timing. The timeliness of surveillance data is complex, and delays in time between the drug seizure, drug testing at the laboratory, and results reporting could be underestimated by researchers. 5) NFLIS geo-location data has limitations. For some laboratories, location information data is not systematically provided. 6) NFLIS-Drug data represent co-reported drugs. Presenting NFLIS-Drug data as "drug combinations" or "drug mixtures" misrepresents what the NFLIS-Drug data show. 7) Varied testing procedures exist at the laboratories. Laboratories also use different criteria to detect drugs in their samples. 8) Overgeneralization and inferential errors can occur. As DEA moves forward with releasing the publicly available data via the Data Query System (DQS), it will be critical for data users to understand that logical inconsistencies and subsequent inferential errors can pose challenges. Knowing the limitations of data sets and considering these limitations during analysis promotes proper comparison of complementary data sets, consistency, accuracy, and usability of the data set.

This study should not be considered a structured systematic literature review and the authors recognize the subjective decisions that were used in drawing our convenience sample. The points included here are intended to serve as illustrative examples to better describe the purposes and limitations of NFLIS data.

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### **NFLIS, Research Methods, Drug Surveillance Reporting**

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## **B203 Project REMEDY: Providing Emerging Drug Data and Materials to the Community**

*Edward Sisco, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Aaron Urbas, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Sara Driscoll, BS, Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, VA; Hunter Haddad, MS, Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, VA; Rebecca Jones, BS, Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, VA; Trinette Spratley, BS, Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, VA; Sherri L. Tupik, BS, Drug Enforcement Administration, Special Testing and Research Laboratory, Dulles, VA*

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**Learning Overview:** After attending this presentation, attendees will understand how the REMEDY project, which is being jointly run by NIST and the DEA, aims to provide reference data and materials of emerging drugs to the community.

**Impact Statement:** This presentation will impact the forensic science community by providing a mechanism for forensic laboratories to obtain reference data and materials, free of charge, for emerging drugs while also assisting with detection of new substances.

The constantly changing emerging drug landscape presents a major need for seized drug chemists to have improved access to standards and reference data in a timely and cost-effective manner. Oftentimes when new substances are encountered in casework, chemists may not be able to identify a substance due to lack of available physical material for comparison purposes. To address this need, Project REMEDY has been established with the goal of providing no-cost reference data and materials to seized drug chemists in such cases where there are no commercial standards available.

Project REMEDY is a joint effort between the National Institute of Standards and Technology (NIST) and the Drug Enforcement Administration Special Testing and Research Laboratory (DEA-SFL1). The goal of the project is to decrease the time lag that can exist between the first appearance of an emerging drug in the United States and the time a viable physical standard is available. Project REMEDY reduces this time lag by rigorously characterizing seized material provided by one of the laboratories within a collaboration network. Characterization of the material occurs independently at both NIST and DEA-SFL1 using a suite of analytical tools including gas chromatography mass spectrometry, liquid chromatography mass spectrometry, nuclear magnetic resonance spectroscopy, direct analysis in real time mass spectrometry, Raman spectroscopy, polarimetry, thermal gravimetric analysis, differential scanning calorimetry, and both solid phase and vapor phase Fourier transform infrared spectroscopy. Once characterized, the resulting data, as well as aliquots or direct forms of the physical material, are made available to the community. Laboratories can then request a portion of the characterized material to better understand the specific responses of the new compound on their instrumentation. The entire process is expected to take three to four weeks.

This presentation will outline the Project REMEDY goals, workflow, and examples of materials that have been characterized. Mechanisms for laboratories to receive and send materials will also be discussed.

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### **Emerging Drugs, Standards, Seized Drug Analysis**

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## **B204 Using DEA’s Forensic Laboratory Drug Chemistry Report Data to Validate the FBI’s Law Enforcement Drug Seizure Data**

*Nick Richardson, PhD\*, RTI International, Research Triangle Park, NC; Wayne Pitts, PhD, RTI International, Research Triangle Park, NC; David Heller, BS, RTI International, Research Triangle Park, NC; Hope Smiley-McDonald, PhD, RTI International, Research Triangle Park, NC; Megan Grabenauer, PhD, RTI International, Research Triangle Park, NC; DeMia Pressley, MS, Drug Enforcement Administration, Springfield, VA*

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**Learning Overview:** After this presentation, attendees will have a broader understanding of the NFLIS-Drug and NIBRS data overall, which can show estimates of drugs in the U.S. illicit drug market.

**Impact Statement:** This presentation highlights how the Drug Enforcement Administration’s NFLIS-Drug data can help validate the Federal Bureau of Investigation’s National Incident-Based Reporting System (NIBRS) data to provide a reliable and valid estimate of the types of drugs seized to measure the illicit drug supply.

Understanding and measuring the illicit drug supply market is critical to effectively responding to the drug epidemic. Data systems to monitor and track the licit drug supply exist, but no standardized data collection system monitors the illicit supply network. Some studies have used law enforcement drug seizures as a proxy by which to monitor the illicit drug supply, but little is known about the quality and validity of law enforcement drug seizure data.

This study compared trends across a 12-month period in 2018 across two important law enforcement drug seizure data sets: NFLIS-Drug and NIBRS. 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. As of January 2021, NIBRS replaced the FBI’s Uniform Crime Reporting Program’s Summary Reporting System. NIBRS captures information on drugs seized by law enforcement, including the suspected drug type and the quantity of the drug seized. Although it is a valuable data set, resource, and tool for researchers interested in examining the relationship between the illicit drug market, virtually nothing is known about the quality of NIBRS drug seizure data.

The primary study goal was to examine how closely the trends from both data sets align from month to month, examining heroin, methamphetamine, and cocaine. Methamphetamine, cocaine, and heroin were used because they have played a key role in fueling the drug epidemic. The study premise is that the drug reports in the NFLIS-Drug data are from drugs tested in a laboratory setting and would yield more accurate and reliable data than would other types of presumptive positive tests that are used by law enforcement in the field and reported in NIBRS.

The authors focused on these drugs seized by law enforcement in Michigan and Tennessee during 2018 based on three NIBRS-related criteria: (1) agency response rate, (2) crime incident coverage, and (3) population coverage. Because NIBRS data are not yet nationally representative, and not all agencies in a state submit data to NIBRS, it was important to ensure that selected states had adequate coverage. For NIBRS, Michigan and Tennessee have greater than 99% coverage for each criterion. All NFLIS-Drug laboratories in Michigan and Tennessee submitted 2018 data.

The results for each of the three drug categories for both states across both data sources exhibit similar longitudinal trends, with the number of drugs seized being higher in NIBRS than in NFLIS-Drug laboratories. Spikes in law enforcement drug seizures in NIBRS are shadowed by spikes in NFLIS-Drug case counts. Similarly, declines in drug seizures in NIBRS mirror drops in NFLIS-Drug cases. These general trends, as well as the few exceptions to these trends, which will be highlighted in the presentation.

Although further work should be done to further validate NIBRS law enforcement drug seizure data, this is the first study to show the correlation between these two important data sets and how they can be used by the forensic, law enforcement, and public health communities to measure the illicit drug market.

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### **NFLIS, NIBRS, Drug Surveillance Reporting**

**B205 The Effect of Modifications to the Core Fentanyl Structure on the Observed Product Ion Spectra**

*Alia Hacker, BS\*, West Virginia University, Morgantown, WV; Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX; Glen Jackson, PhD, West Virginia University, Morgantown, WV*

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**WITHDRAWN**

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## B206 An Evaluation of the Physical and Chemical Variation in Tablets Found to Contain Fentanyl

*Emily Lockhart, MS\*, U.S. Drug Enforcement Administration, Dulles, VA; James Jordan, PhD, U.S. Geological Survey, Reston, VA*

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**Learning Overview:** Attendees will gain a fundamental understanding of the physical characteristics and chemical properties of clandestine fentanyl tablets.

**Impact Statement:** This presentation will impact the forensic science community by presenting data from the testing of individual tablets within batches of seized clandestine fentanyl tablets and investigating sources of variation within seizures and across different seizures as potential indicators for routes of introduction into the illicit US opioid market.

Fentanyl in the United States' illicit drug market has been a prolific concern since circa 2014. According to internal studies by the DEA Intelligence Division, which included data from the Center for Disease Control and Prevention (CDC), the prevalence of illicit tablets containing fentanyl available to the public is a national health threat as deaths involving synthetic opioids continue to increase.<sup>1</sup> The National Forensic Laboratory Information System (NFLIS) reported a 12% increase in fentanyl-related incidents in 2019 when compared to the previous year.<sup>1</sup>

Illicit tableting operations occur domestically and are especially prolific in Mexico. A report from the Partnership for Safe Medicines states that as of October 2020, all 50 US States have reportedly identified fentanyl in clandestine tablets.<sup>2</sup> Tablets bearing the mark "M 30" (normally indicative of 30 milligram oxycodone tablets) are consistently observed in the illicit market.<sup>1</sup> Other tablet markings such as "A 215" are also seen. Mexican authorities have reported an increase in illicit opioid tableting operations assuring that fentanyl will continue to be observed in the illicit market.<sup>1</sup>

The goal of this research is to characterize individual clandestine fentanyl tablets from physical and chemical perspectives. Analysis of individual tablets versus bulk composites provides valuable data pertaining to within seizure and seizure-to-seizure variation which is necessary to gain insight to the level of sophistication in illicit manufacturing operations. Physical characterization included measurements of sample mass, diameter, thickness, and markings. Additionally, high resolution photos of the tablets were collected using a camera coupled to a microscope. Tablet color (normally a subjective call) was more fully characterized by fiber-optic spectrophotometry in diffuse reflectance mode. Chemical characterization included qualitative identification and quantitation of all components via nuclear magnetic resonance (NMR) and profiling of elemental impurities using X-ray fluorescence (XRF) and/or laser ionization breakdown spectroscopy (LIBS).

### Reference(s):

- <sup>1.</sup> Drug Enforcement Administration 2020 National Drug Threat Assessment (March 2021).
- <sup>2.</sup> Partnership for Safe Medicines. *Illegal Pill Presses: 2021 Update* (May 2021) [www.safemedicines.org/policymakers-media/illegal-pill-presses-2021-update](http://www.safemedicines.org/policymakers-media/illegal-pill-presses-2021-update).

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### Seized Drugs, Fentanyl, Tablets

## B207 An Analysis of Fentalogs and Illicit Fentanyl Vapor Profiles

*Stephanie Vaughan, PhD\**, Naval Research Laboratory, Washington, DC; *Ashley Fulton, PhD*, Naval Research Laboratory, Washington, DC; *Lauryn DeGreeff, PhD\**, Formerly Naval Research Laboratory, Washington, DC

**Learning Overview:** After attending this presentation, attendees will understand the methods used for headspace analysis, as well as the relationship between pharmaceutical-grade fentanyl, fentalogs, and illicit fentanyl.

**Impact Statement:** This presentation will impact the forensic science community by identifying target analytes that can be used to detect fentanyl in the vapor phase.

Fentanyl is a Schedule II synthetic opioid that possesses strong euphoric and analgesic properties, as well as high potency. With its immense potency and availability, fentanyl poses great danger to users and law enforcement personnel. The risk of overdose is extremely high, with as little as 2 mg milligrams capable of a lethal dose. In 2019, there were 36,359 synthetic opioid deaths, with most caused by fentanyl or fentanyl-related substances.<sup>1</sup> Availability of fentanyl has been consistently on the rise with a 180% increase of seizures from 2016 to 2019.<sup>2</sup> Due to this hazard, most agencies advise personnel to avoid direct handling of fentanyl or related materials.

To avoid the dangers of fentanyl in the field, a non-contact detection method would be ideal; however, due to the low vapor pressure of fentanyl it is not likely to be detected in the vapor phase. Thus, it is necessary to identify other compounds in the headspace that can be used as target analytes for vapor detection. In this regard, the vapor profile of fentanyl can be determined by identifying volatile organic compounds (VOCs) found in the headspace that are specific to fentanyl. This presentation will describe the research used to determine the vapor profiles of pharmaceutical-grade fentanyl, fentalogs (fentanyl analogs), and illicit fentanyl, as well as identification of target analytes for vapor detection of fentanyl.

A solid phase microextraction (SPME) coupled with gas chromatography and mass spectrometry (GC-MS) method was used to assess the vapor profiles. Several semi-VOCs and VOCs were identified in the headspaces, with each having a unique vapor profile. A comparative analysis of all vapor profiles was completed, which included a total of 20 illicit fentanyl samples and fentalogs. The analysis revealed two targets of interest for detection of fentanyl and related substances in the vapor phase: N-phenylpropanamide (NPPA) or N-phenethyl-4-piperidone (NPP). It was determined that NPPA is formed through fragmentation of fentanyl through  $\beta$ -elimination, while NPP is a constituent of the synthetic route for fentanyl and many fentalogs. The identification of NPPA or NPP in 75% of these samples is an essential component in the development of a non-contact vapor detection method for fentanyl.

### Reference(s):

- <sup>1</sup> National Institute on Drug Abuse. *Overdoses Death Rates*. 29 January 2021. [Online]. Available: <https://www.drugabuse.gov/drug-topics/trends-statistics/overdose-death-rates>. [Accessed May 2021].
- <sup>2</sup> Drug Enforcement Administration; Special Testing and Research Laboratory. *Annual Emerging Threat Report*. 2019.

### Fentanyl, SPME, Vapor Detection

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## **B208 The New National Institute of Standards and Technology/National Institute of Justice (NIST/NIJ) Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) Data Interpretation Tool (DIT)**

*Arun Moorthy, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Stephen Tennyson, BS, National Institute of Standards and Technology, Gaithersburg, MD and University of Maryland, College Park, MD; Ruthmara Corzo, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending this presentation, attendees will know how to employ the new NIST/NIJ Direct Analysis in Real Time Mass Spectrometry (DART®-MS) Data Interpretation Tool (DIT) for seized drug analysis.

**Impact Statement:** The NIST/NIJ DART®-MS DIT is a freely available and open-source software tool developed in collaboration with practicing local, state, and federal forensic laboratories doing seized drug analysis. This tool simplifies DART®-MS analysis of seized drugs for forensic chemists, and the open-source nature of the software allows for further customization and expansions.

DART®-MS is an analytical chemistry technology being increasingly employed in forensic applications.<sup>1</sup> This form of mass spectrometry rapidly yields rich structural information about an analyte with minimal sample preparation. The challenge with DART®-MS data, much like other data generated with high throughput technologies, lies in the data interpretation; this is especially true when the analyzed samples are multi component mixtures like seized drug evidence.

The NIST/NIJ DART®-MS DIT is a freely available and open-source software tool developed to support the interpretation of DART®-MS data. The NIST/NIJ DART®-MS DIT can be used to view reference mass spectra from DART®-MS spectral libraries, search query DART®-MS mass spectra of mixtures against reference libraries using the Inverted Library Search Algorithm, and generate printable reports from search results.<sup>2,3</sup> Several of the features, including the formatting of generated reports, were iteratively designed with input from local, state, and federal forensic practitioners, ensuring that the program is intuitive and usable for the expected users.

This presentation will discuss the process of creating the NIST/NIJ DART®-MS DIT, including how feedback was solicited and incorporated through the development process, as well as demonstrate how new users can employ DART®-MS and this new software to analyze seized drug evidence.

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

1. Sisco, E.; Forbes, T.P. Forensic applications of DART®-MS: A review of recent literature. *Forensic Chemistry*. 2020; 100294.
2. Sisco, E.; Moorthy, A.S.; Watt, L.M. Creation and Release of an Updated NIST DART®-MS Forensics Database. *Journal of the American Society for Mass Spectrometry*. 2021; 32(3): 685-689.
3. Moorthy, A.S.; Sisco, E. A new library-search algorithm for mixture analysis using DART®-MS. *Journal of the American Society for Mass Spectrometry*. 2021; 32(7): 1725-1734.

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### **DART®-MS, Software, Seized Drugs**



## B209 Best Buds—Ambient Ionization Coupled with Mass Spectrometry for the Forensic Analysis of Cannabinoid-infused Complex Matrices

*Megan Chambers, BS\*, University at Albany - SUNY, Albany, NY; Rabi Musah, PhD, University at Albany - SUNY, Albany, NY*

**Learning Overview:** In this presentation attendees will gain an understanding of how ambient ionization coupled with mass spectrometry can be utilized for the rapid analysis of various Cannabis-derived and cannabinoid-infused samples, including plant material, edibles, beverages and personal-care products.

**Impact Statement:** This presentation will impact practices in the fields of forensic science and criminal justice by providing protocols for the rapid detection and quantification of cannabinoids in complex matrices, as well as an approach to differentiate hemp and marijuana varieties of *Cannabis sativa*.

Exploration of new methods for the analysis of Cannabis-derived materials has exploded in recent years. This is primarily a result of the ever-evolving sample types that require analysis. In the field of forensic science, crime laboratories can receive Cannabis and cannabinoids as evidence in a variety of forms, and the complexity of these matrices can cause numerous problems when analyzed by traditional forensic methodologies. However, also in recent years, a technique termed direct analysis in real time – high-resolution mass spectrometry (DART<sup>®</sup>-HRMS) has been utilized in several areas of forensic science, including seized drugs. This technique confers several potential benefits that can be leveraged in the analysis of Cannabis evidence, as described herein.

The primary difficulty when it comes to analyzing edibles infused with cannabinoids is how complex the matrices are. In this study, a variety of cannabinoid-infused food and beverages were prepared in-house, while personal care-products were purchased from local vendors. The two primary cannabinoids of interest are psychoactive  $\Delta^9$ -tetrahydrocannabinol (THC) and non-psychoactive cannabidiol (CBD). When screened by DART<sup>®</sup>-HRMS, samples containing one or both of these cannabinoids produced a mass spectrum containing a peak at  $m/z$  315, which is consistent with the protonated mass  $[M+H]^+$  of THC and CBD. Control samples (blank samples that do not contain cannabinoids) were also analyzed in this study. Despite the complexity of their matrices, none of the controls exhibited peaks that overlapped with those consistent with cannabinoids. The successful detection of cannabinoids in complex matrices prompted research towards the development of quantification protocols using DART<sup>®</sup>-HRMS, including the quantification of CBD in traditionally challenging edible matrices (i.e., gummies, chocolates). Extraction protocols and the DART<sup>®</sup>-HRMS method for quantification are being optimized for integration into current forensic laboratory workflows.

Another challenging aspect of analyzing Cannabis evidence is the differentiation of hemp and marijuana plant material, which are two varieties of *Cannabis sativa*. Although both varieties contain THC, they differ in the amount of this molecule that is present. Federal law currently states that *C. sativa* that contains  $>0.3\%$  THC is marijuana, while plant material that contains  $\leq 0.3\%$  THC is hemp. This definition imposes severe challenges on crime labs. Therefore, in addition to the detection and quantification of cannabinoids, a DART<sup>®</sup>-HRMS method combined with statistical analysis was developed for the rapid differentiation of hemp and marijuana plant material. Marijuana samples from DEA-registered suppliers and hemp plant material from licensed vendors were obtained and analyzed by DART<sup>®</sup>-HRMS. Preliminary statistical analysis of the small-molecule profiles of these samples revealed the potential for differentiating these varieties by DART<sup>®</sup>-HRMS. These results prompted the application of advanced statistical processing to the data, which revealed diagnostic  $m/z$  values for distinguishing between hemp and marijuana with a high level of certainty. The identities of several  $m/z$  values have been confirmed, while the identification of the remaining masses is currently underway.

DART<sup>®</sup>-HRMS has demonstrated successful applications to the investigation of seized *C. sativa* plant material and derived products. Overall, the methods described would greatly improve forensic science and criminal justice practices in the United States.

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### ***Cannabis Sativa*, Cannabinoids, Edibles**

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## **B210 A Retrospective Review of Hemp/Marijuana Decision Point Assays in Operational Laboratories**

*James Miller, MA\*, Houston Forensic Science Center, Houston, TX; Kay McClain, BS, Harris County Institute of Forensic Sciences, Houston, TX; Charles Cline, BS, Texas Department of Public Safety Crime Laboratory Service, Tyler, TX; Ya-Chih Cheng MS, Sam Houston State University, Huntsville, TX; Sarah Kerrigan PhD, Sam Houston State University, Huntsville, TX*

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the practical and scientific considerations associated with the deployment of decision-point assays for the differentiation of hemp from marijuana in a high-throughput operational setting.

**Impact Statement:** The presentation will impact the forensic science community by highlighting important concepts and considerations associated with the use of decision-point assays related to hemp/marijuana differentiation.

Decision-point assays are frequently used to differentiate illegal marijuana from legal or commercial hemp in many jurisdictions. We previously reported an interlaboratory validation at a 1%  $\Delta 9$ -tetrahydrocannabinol ( $\Delta 9$ -THC) administrative threshold using gas chromatography/mass spectrometry (GC/MS), deuterated internal standard and dual selected ion monitoring (SIM)/scan acquisition. This threshold far exceeds the legal threshold of 0.3%  $\Delta 9$ -THC by dry weight. Although multi-agency collaborations and interlaboratory validations are extremely beneficial, they do not supplant the need for there to be a full, independent, and rigorous validation. Prior to deployment, the method was fully validated in accordance with published standards in seized drug analysis. This included limit of detection, selectivity, precision, accuracy, dilution integrity, carryover, extract stability, decarboxylation efficiency, measurement uncertainty, and potential interference from cannabidiol (CBD). Certified reference materials and secondary controls were employed during validation and routine casework.

In this presentation we share data and the experience of three independent agencies and fifteen laboratories following deployment of the method for over one year. Practical challenges, maintenance, sample throughput, reporting practices, operational workflow and resource sharing are discussed.

Using the decision-point approach, the relative peak area (RPA) of the unknown ( $\Delta 9$ -THC/ $\Delta 9$ -THC- $D_3$ ) is measured relative to the positive control at the administrative cutoff (1%  $\Delta 9$ -THC by weight). This ratio of RPAs establishes the decision-point ratio (DPR) whereby samples with a DPR of 1.0 or more exceed the reporting cutoff. Although not quantitative in nature, the spread of decision-point ratios among exhibits provides an estimate of how close a large population of seized drug specimens across laboratories and jurisdictions are to the administrative cutoff. Data between laboratories are correlated, and interesting (outlier) data are discussed.

Differences in instrument performance and strategies for routine maintenance are addressed. Workflow issues and the use of a customized macro among laboratories are presented. Finally, reporting strategies that reflect differences in laboratory approach, jurisdictional and policy-based preferences are also discussed. Data and experiences across multiple agencies highlight the utility and efficiency of the approach, while maintaining a strict regimen for instrument maintenance and performance monitoring.

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### **Hemp, Marijuana, GC/MS**

## B211 Factors Influencing the *In Situ* Formation of Psychoactive Cannabinoids from Cannabidiol During GC-MS Analysis

*Student Ya-Chih Cheng, MS\*, Sam Houston State University, Huntsville, TX; Sarah Kerrigan, PhD, Sam Houston State University, Huntsville, TX*

**Learning Overview:** After attending this presentation, attendees will understand the potential for CBD to  $\Delta$ 9-THC conversion in the GC inlet and strategies for mitigation.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness regarding the potential for in-situ formation of psychoactive cannabinoids, and the role of instrument conditions, preventive maintenance, and liner selection to mitigate issues.

*Cannabis sativa* (*C. sativa*) can be broadly classified as hemp or marijuana. While marijuana yields a higher concentration of the psychoactive compound,  $\Delta$ 9-tetrahydrocannabinol ( $\Delta$ 9-THC), industrial or commercial hemp products are typically rich in cannabidiol (CBD). Gas chromatography/mass spectrometry (GC/MS) is the most widely used instrumental technique for the identification of  $\Delta$ 9-THC in seized plant materials. As a consequence, it is commonly deployed for the differentiation of hemp from marijuana in the United States and Europe. However, the potential for CBD conversion to  $\Delta$ 9-THC and other cannabinoids exists under certain conditions. This conversion is exploited in e-cigarettes due to their extremely high operating temperatures. Although temperatures in the GC inlet are typically much lower than e-cigarette devices, the potential for conversion must be carefully evaluated during GC/MS method development and validation. Other mitigating factors, such as active sites, and absence of routine maintenance could potentially exacerbate formation.

We previously developed and validated a GC/MS assay to differentiate hemp from marijuana using an administrative threshold of 1%  $\Delta$ 9-THC by weight. Plant extracts containing CBD concentrations equivalent to 50% (w/w) were evaluated during the validation. Instrumental conditions were selected to minimize in-situ formation. Anecdotally, differences in assay performance were observed following routine maintenance.

In this study, seven GC liners with different deactivation chemistries and geometries were evaluated. The effect of deactivation chemistry was compared using four cyclosplitter liners with standard deactivation, base deactivation, Siltek deactivation, and premium deactivation. The influence of liner geometry was also evaluated using liners with the same surface deactivation chemistry. In order to produce detectable  $\Delta$ 9-THC, no preventive maintenance was performed (including column maintenance or source cleaning) for several months.

Significant differences in CBD to  $\Delta$ 9-THC production were observed between liner type. In many instances it was not possible to produce sufficient  $\Delta$ 9-THC to meet reporting criteria (typically ion ratios). Three of the liners evaluated were capable of producing reportable  $\Delta$ 9-THC from CBD. Although inlet temperature had been previously optimized (250°C), the effect of temperature (230 – 290°C) was also investigated. Due to extreme differences in abundance, temperature dependence was observed for some liners but not others. However, a two-way ANOVA confirmed inlet temperature had a significant effect on  $\Delta$ 9-THC production at  $p=0.05$  [ $F(3, 32)=16.04, p<0.0001$ ]. ANOVA was also used to show that differences between instruments operating under the same conditions were significantly different [ $F(1, 32)=244.31, p<0.0001$ ]. The results confirmed that the original deactivated liner that was used for the validation was resistant to the formation of  $\Delta$ 9-THC under the operating conditions of the assay. However, some liners, including those packed with glass wool were more susceptible to in-situ production of  $\Delta$ 9-THC. Using the least favorable liner, CBD to  $\Delta$ 9-THC was found to be 20 times higher. Moreover, using this liner it was also possible to identify  $\Delta$ 8-THC from CBD at high concentrations (40%).

Under forceful conditions and in the absence of routine preventive maintenance, measurable quantities of  $\Delta$ 9-THC can be produced in-situ. Careful selection of instrumental conditions, liner selection and routine preventive maintenance can mitigate this. Differences between instruments highlight the need for full, independent and rigorous validation, as well as appropriate safeguards during routine analysis.

### Marijuana, Hemp, CBD

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## B212 Analysis of Cannabis Plant Materials by Infrared Spectroscopy for Differentiating Hemp and Marijuana

*Aaron Urbas, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD; *Ewelina Mistek-Morabito, PhD*, University at Albany, SUNY, Albany, NY; *Igor Lednev, PhD*, University at Albany, SUNY, Albany, NY; *Walter Wilson, PhD*, National Institute of Standards and Technology, Gaithersburg, MD; *Melissa Phillips, PhD*, National Institute of Standards and Technology, Gaithersburg, MD

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**Learning Overview:** The goal of this presentation is to provide forensic scientists with an overview of the potential utility of mid-infrared and near-infrared spectroscopic analysis for the rapid evaluation of Cannabis plant materials for distinguishing hemp and marijuana.

**Impact Statement:** This presentation will impact the forensic community by summarizing near-infrared (NIR) spectroscopic analysis of cannabis conducted on a benchtop FTIR system as well as an evaluation of several commercially available portable cannabis analyzers, based on IR (ATR) or NIR (reflectance) spectroscopy, for the quantification of THC and differentiation of Cannabis samples as hemp or marijuana.

Identification and quantification of seized drugs are important aspects of current forensic practices. With the passage of the 2018 Farm Bill, the analysis of cannabis samples has become more challenging for forensic practitioners. There is an urgent need for rapid and reliable methods to differentiate between legal hemp and illegal marijuana samples. There are no taxonomical differences between hemp and marijuana and current field tests to distinguish them are limited. The term hemp is used to refer to strains of cannabis that have low levels of tetrahydrocannabinol (THC). However, the new federal legislation defined legal hemp as containing 0.3% or less total-THC, estimated as decarboxylated- $\Delta^9$ -THC, on a dry weight basis. This designation requires that forensic practitioners quantify the total-THC content in seized cannabis samples.

In this work, we explored the use of near-infrared (NIR) reflectance spectroscopy to analyze a wide variety of ground cannabis plant materials on a benchtop FTIR spectrometer system. A total of 85 samples were analyzed for different aspects of the investigation. The data was analyzed using multivariate analysis methods to both qualitatively discriminate between cannabis strains/samples designated as hemp or marijuana and to develop quantitative models for predicting total-THC content. Classification models, based on partial least-squares discriminant analysis (PLS-DA), showed clear differentiation between hemp and marijuana strains. However, numerous hemp samples were found to contain levels of THC above 0.3% based on reference THC content obtained using a high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for a large subset of the subsamples. Partial least-squares (PLS) regression models were developed on this sample set to quantify total-THC content and determine whether sufficient accuracy could be obtained for forensic or regulatory purposes.

In addition to our own spectroscopic investigations, a subset of cannabis samples was evaluated on four commercially available small footprint, portable cannabis analyzers based on either mid-IR (ATR) or NIR reflectance spectroscopy. These systems came with built-in calibrations that provided quantitative estimates of total-THC content as well as other cannabinoids (e.g., CBD). The results from these commercial systems were compared to the total-THC values obtained from the reference HPLC-UV method and will also be presented.

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### Cannabis, Infrared Spectroscopy, Seized Drug Analysis

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## **B213 The National Institute of Standards and Technology (NIST) Cannabis Quality Assurance Program (CannaQAP): A Tool for Improving Quantitative Measurements in Cannabis**

*Melissa Phillips, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Walter Wilson, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** To increase understanding about CannaQAP, the impact participation in a QAP can have on a forensic laboratory, and consideration of how future QAPs could be instrumental in standardizing forensic testing approaches.

**Impact Statement:** CannaQAP is the first large-scale interlaboratory study to investigate quantitative measurements of cannabinoids and other constituents in Cannabis. CannaQAP, and future similar programs, will generate a wealth of information for the forensic community.

Accurate and precise measurements are critical for enforcement of laws and regulations. Many tools are available to help laboratories critically evaluate performance, including standard methods, reference materials, and interlaboratory studies. The National Institute of Standards and Technology (NIST) uses interlaboratory studies as a tool to establish scientific validity of a method, to uncover method specific results, to evaluate fitness-for-purpose of candidate reference materials, and to gather multi-laboratory testing data to support method standardization.

The NIST Cannabis Quality Assurance Program (CannaQAP), an ongoing educational interlaboratory comparison, launched in 2020 as a mechanism to improve the comparability of analytical measurements in forensic and Cannabis (hemp and marijuana) testing laboratories. CannaQAP operates similarly to traditional proficiency testing schemes but without pass/fail implications. Results from CannaQAP participants are evaluated with respect to the consensus of submitted results as well as to NIST results, and all studies are summarized in publicly available NIST Reports. Exercise 1 of CannaQAP offered opportunities for participants to demonstrate proficiency in the determination of cannabinoids in two hemp oils. Exercise 2 offered studies for determination of cannabinoids in Cannabis materials both above and below the US federal limit for total THC (0.3%), as well as moisture and toxic elements in hemp plant materials. Exercise 3 repeated studies previously offered in Exercises 1 and 2 as an opportunity for laboratories to demonstrate improved performance. Over 200 laboratories have participated in CannaQAP, including Cannabis product developers, contract research organizations, regulators, and forensic laboratories. Involvement of stakeholders from across the Cannabis community has contributed to the impact of CannaQAP, allowing laboratories from different sectors to learn from one another's experience.

This presentation will summarize the goals and impact of CannaQAP, including results from the first three exercises. These studies have revealed the overall performance of Cannabis testing laboratories in quantitative evaluation of cannabinoids, toxic elements, and moisture in Cannabis plant and oil materials. In addition, method information collected via CannaQAP participation has identified potential areas for improvement of in-house methods and directions for standard methods currently in development. Future studies of CannaQAP will engage additional standards development organizations and include studies for other Cannabis-containing samples, building upon lessons learned and providing a tool for laboratories to demonstrate comparability.

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### **Cannabis, Seized Samples, Δ9-THC**

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## **B214 Testosterone as an Internal Standard in Qualitative Decision-Point Assays for the Differentiation of Hemp From Cannabis: Optimization and Limitations in High CBD Matrices**

*Jose Gonzalez, BS\*, Los Angeles Police Department, Los Angeles, CA*

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**Learning Overview:** An attendee can expect to learn how to best optimize and understand the limitations of Testosterone as an internal standard in qualitative decision-point assays used to differentiate Hemp from Cannabis.

**Impact Statement:** My presentation will present information which will shed light on chromatographic and sample preparation approaches to handle sample matrices which have proven difficult to analyze in the past where testosterone was/is utilized as an internal standard.

Background: Traditionally government laboratories at the local, state, and federal level tasked with analyzing plant material for the presence of Cannabis used a two-tier testing approach: One a morphological examination and the other a chemical color test known as the Modified Duquenois-Levine test. These controlled substance laboratories have historically been focused on qualitative work- the identification and weight of a controlled substance. Quantitatively determining the amount of controlled substances has typically been left to toxicologists where such analysis is routine. However, with the passage of the Farm Bill in 2018, control substance laboratories now had to come up with methodologies to differentiate Hemp from Cannabis.

There are various ways to tackle the issue ranging from spectrophotometric techniques to high pressure liquid chromatography to name a few. In an effort to provide a solution to the analytical challenge, some laboratories settled on a decision-point assay technique which relies on one of the techniques utilizes a common instrument in controlled substance laboratories - a gas chromatograph/mass spectrometer (GC/MS).<sup>1,2</sup> Decision-point assays look at the ratio of a known amount of delta-9 THC to the amount of delta-9-THC in the sample utilizing an internal standard technique.

This work looks at the use of Testosterone<sup>1</sup> as an internal standard in various sample matrices. The challenge of utilizing Testosterone in such matrices will be discussed as well as the inhibition of Testosterone in certain high CBD profile samples.

**Reference(s):**

- <sup>1.</sup> Method Validation Report for the Identification and Quantitative Threshold Testing of Tetrahydrocannabinol (THC) in Cannabis. Palm Beach County Sherriff Office.
- <sup>2.</sup> Qualitative Analysis of Cannabinoids by Gas Chromatography. Drug Enforcement Agency, Office of Forensic Sciences.

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**Cannabis, Hemp, Testosterone**

**B215 Distinguishing Hemp and Marijuana Using a Portable Mid-Infrared Spectrometer**

*Sandra Rodriguez-Cruz, PhD\**, DEA Special Testing and Research Lab, Dulles, VA; *Michael Weaver, BS*, DEA Special Testing and Research Lab, Dulles, VA

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**WITHDRAWN**

## C1 The Validation of Imagery Enhancement Tools

*James Zjalic, MSc\*, Verden Forensics, Birmingham, Birmingham, United Kingdom; Jake Harrison, BSc, Verden Forensics, Birmingham, Birmingham, United Kingdom*

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of validating tools relied upon as part of a wider method and the need to exercise caution when selecting said tools. Attendees will be presented with the methodology, results, and conclusions from research performed in relation to the validation tool testing of imagery enhancement tools that are marketed at different sectors (including forensics and graphic design) and thus fall into different price brackets.

**Impact Statement:** The study aims to increase awareness of the requirement for tool validation and to assist examiners in making informed decisions about the tools they use for the enhancement of images.

The validation of methods is an essential component of forensics to aid in improving consistency and is also one of the core requirements of the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025:2107 standard.<sup>1,2</sup> This reliability is based on two principles: accuracy (will the method/tool produce the expected [and thus reliable] results?), and precision (will the method/tool produce the same results repeatedly?). In order to validate a method, it is vital that the tools used within the said method are reliable, and thus, the validation of tools forms a vital part of the overall validation exercise.

During validation of the imagery enhancement method, results were obtained using a tool that is marketed specifically for use in forensics. Curiosity then drove further tool testing into the consistency of results against other imagery enhancement solutions. As the focus of the research is tool validation, and not the performance of specific tools, all software tested has been anonymized.

The overarching research questions were: (1) do the tools apply processes as expected?, and (2) what is the degree of inter-variability between the results of processes applied by different software?

Hypotheses stemming from such are that the processes would be applied as expected, and thus the inter-variability would not significantly impact the overall result of the method. This is based on the premise that imagery enhancement tools would apply similar processes to the imagery based on fundamentals of image processing.

In order to test the proposed hypothesis, the same images were processed using the same settings with each tool. The processes applied included: cropping, look up table adjustments, brightness, contrast, sharpening, and rescaling.

### Reference(s):

- <sup>1.</sup> ISO/IEC. ISO 17025:2017 General requirements for the competence of testing and calibration laboratories. 2017.
- <sup>2.</sup> The Forensic Science Regulator. Method Validation in Digital Forensics FSR-G-218. No. 1, p. 45.

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### Digital Imagery, Validation, Digital Tools



### C2 Score-Based Likelihood Ratios for Camera Device Identification Using Cameras of the Same Brand for the Alternative Device Population

Stephanie Reinders, PhD\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Iowa State University, Ames, IA; Danica Ommen, PhD, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Iowa State University, Ames, IA; Alicia Carriquiry, PhD, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Iowa State University, Ames, IA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the benefits and limitations of using score-based likelihood ratios to quantify the weight of evidence in camera device identification and how the alternative cameras used in the analyses can affect the rates of misleading evidence.

**Impact Statement:** A digital image (questioned image) is involved in a crime and investigators want to determine whether the image was captured by a person of interest's camera. This presentation will impact the forensic science community by explaining how score-based likelihood ratios can quantify the strength of the evidence in this scenario. The presentation will also explore how the set of alternative cameras used in the analyses can affect the results.

Score-based likelihood ratios are a statistical method for quantifying the weight of evidence and have been used in many areas of forensics, including camera device identification.<sup>1-3</sup> Small sensor imperfections caused during manufacturing, called photo response non-uniformity, leave identifying features, called a camera fingerprint, in the images that a camera takes.<sup>4</sup> The sample correlation measures the similarity (or dissimilarity) between the camera fingerprint from the person of interest's camera and the camera fingerprint in the questioned image. On its own, it is difficult to know how to interpret this score. Is a score of 0.25 evidence that the questioned image originated from the person of interest's camera? What about a score of 0.5? To make sense of the score, it is compared with two different reference sets of scores: matching and non-matching. Matching scores are sample correlations between two fingerprints known to come from the person of interest's camera. Non-matching scores are sample correlations between two fingerprints known to come from two different cameras. An alternative set of cameras that does not include the person of interest's camera is used to build the set of non-matching scores.

It turns out that researchers have not agreed upon a best method for constructing the alternative population for score-based likelihood ratios.<sup>5,6</sup> Recently, researchers calculated score-based likelihood ratios for camera device identification using 48 cameras representing 26 models.<sup>7</sup> This present research explores whether the rates of misleading evidence can be decreased by restricting the alternative device population to cameras of the same brand as the person of interest's camera.

#### Reference(s):

1. A. Nordgaard; T. Höglund. Assessment of approximate likelihood ratios from continuous distributions: A case study of digital camera identification. *Journal of forensic sciences*, vol. 56, no. 2, pp. 390-402, 2011.
2. W. van Houten; I. Alberink; Z. Geradts. Implementation of the likelihood ratio framework for camera identification based on sensor noise patterns. *Law, Probability and Risk*, vol. 10, no. 2, pp. 149-159, 2011.
3. S. Reinders; L. Lin; W. Chen; Y. Guan; J. Newman. Score-based likelihood ratios for camera device identification. In *Electronic Imaging*, Burlingame, CA, 2020.
4. J. Lukas; J. Fridrich; M. Goljan. Determining digital image origin using sensor imperfections. In *Image and Video Communications and Processing 2005*, 2005.
5. C. Champod; I.W. Evett; G. Jackson. Establishing the most appropriate databases for addressing source level propositions. *Science & Justice*, vol. 44, no. 3, pp. 153-164, 2004.
6. D.M. Ommen; C.P. Saunders. Building a unified statistical framework for the forensic identification of source problems. *Law, Probability and Risk*, vol. 17, no. 2, pp. 179-197, 2018.
7. S. Reinders. *Statistical Methods for Digital Image Forensics: Algorithm Mismatch for Blind Spatial Steganalysis and Score-based Likelihood Ratios for Camera Device Identification*. Doctoral Dissertation, Iowa State University, 2020.

#### Camera Identification, Score-Based Likelihood Ratios, Digital Evidence

## C3 The Influence of Compression on Forensic Deepfake Detection

*Meike Kombrink, MFS, Netherlands Forensics Institute, The Hague, South Holland, Netherlands; Zeno Geradts PhD\*, Netherlands Forensics Institute, The Hague, South Holland, Netherlands and University of Amsterdam, Amsterdam, North Holland, Netherlands*

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**Learning Overview:** After attending this presentation, attendees will have learned more on deepfake detection algorithms in relation to compression algorithms used.

**Impact Statement:** This presentation will impact the forensic science community by giving more direction of research, as well as handling issues with deepfakes on social media.

Many videos are shared on online platforms every day. Yet it is becoming increasingly difficult to determine the authenticity of these videos due to the increased popularity and availability of deepfake creation software. Deepfake videos are videos that have been manipulated using deep learning. Although many different types of deepfakes exist, the current, most popular type of deepfake is a face swap between a target person and an individual in a video. The quality of deepfake videos has improved drastically, to the point where deepfake videos and genuine videos are becoming harder to distinguish with the human eye. While the analysis of single, high-impact videos for evidence of manipulation is possible, this can only be performed properly by experts (making this method of detection limited in its applicability) for whom the process is time consuming. The limited number of experts available to distinguish between deepfakes and genuine videos means that we cannot analyze all videos that are uploaded daily given this current speed of detection. In order to review each of the hundreds of thousands of videos uploaded to internet or social media platforms every day, automation of the process is needed.

Recently, the automated detection of deepfake videos has experienced a surge of interest, which led to a lot of improvements in the performance of algorithms to detect deepfakes. Yet these improvements have been made on either largely abstracted datasets or on datasets without annotation of complexities. One such complexity that always occurs is compression, which launders manipulation traces from images. As many deepfakes are shared on social media, where any upload will always first be compressed, it is vital to understand the influence of compression on the detection of deepfake videos. Therefore, this research will investigate the influence of compression on the ability of two promising detection algorithms to distinguish between deepfake videos and genuine videos. The results of this research suggest that the effect of compression on the detection of deepfake is positive at first, but turns negative when too much compression is added. The studied algorithms show almost identical effects to compression. The study also suggests similar effects of compression are seen for H.265 and AV1, though this result is only gathered on a limited dataset and needs more research before any conclusions should be drawn. Also, other approaches such as lip-reading with Artificial Intelligence (AI) have been tested, and the combination of different methods for detection both for audio, speaker and video artifacts seem to be the way forward.

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### Compression, Deepfake, Video Manipulation

## C4 The Performance of Automated Age Assessment Methods in Images of Subadults

Petra Urbanova, PhD\*, Masaryk University, Brno, CZ, Czech Republic; Pavel Bednar, Masaryk University, Brno, CZ, Czech Republic

**Learning Overview:** After attending this presentation, attendees will be acquainted with the basic principles on which artificial intelligence and machine learning algorithms are based for age assessment in images. Attendees will be acquainted with the performance of algorithms employing neural networks, convolutional neural networks, and support vector machines when tested on a sizable biometric database of subadult faces.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into the current state of the art in forensic image age assessment by comparing the performance of machine learning methods.

Assessing the age of individuals is an important forensic task. There are numerous situations in which an individual's age needs to be estimated or verified—from everyday interactions (age-restricted access or purchases), to circumstances in which an age limit bears crucial legal consequences, to motivations for commercial profits (age-adaptive marketing strategies). While age estimation considers age as a continuum or ordinal variable, age verification is purely a classification task, which determines whether a person falls above or below a given age threshold. Currently, forensic casework requiring age assessment is mostly performed using images (photographs, video stills) and real-time videos or recordings (Closed-Circuit Television [CCTV] or webcam), and there are ongoing attempts to develop age assessment algorithms that would perform most accurately. However, to date, most of the studies have ignored the fact that the estimation error for subadult individuals varies significantly depending on the age, ancestry, and sex of the individual.

The present study explores the performance of state-of-the-art automated algorithms for image age estimation in subadults. It aims to explore the limitations of such tasks by using different types of input data and machine learning methods.

The studied sample consisted of 1,164 individuals of predominantly European descent, aged between 6 and 19 years (mean 11.13 years). Image data were sampled from the Facial Identification Software (FIDENTIS) 3D Face Database. For each 3D image, 30 feature points were manually collected. In addition, a subset of 22 landmarks was automatically acquired by running an in-house algorithm.<sup>1</sup> To test the image-based algorithms, the 3D images were converted to 2D color images that varied in viewpoints and technical parameters to mimic the constantly changing acquisition conditions (image resolution, pose, object-to-camera distance, focal length). The conversion was carried out in a non-commercial converter.<sup>2</sup> To train the automated systems, the dataset was divided into a training (75%) and a test (25%) subset. Subsequently, (Convolutional) Neural Networks (CNNs, NNs) and Support-Vector Machines (SVM) were tested on a pooled dataset and separately on males and females. For 3D feature points, an NN architecture consisting of 90 neurons, 2 hidden layers with 200 and 50 neurons, respectively, and SVM were employed. For each algorithm, training and testing was repeated 50 times. The CNN architecture was based on pre-trained ResNet18 and VGG-16 networks. Ultimately, a subset of 100 images, randomly selected from the converted images, was evaluated by 100 participants trained in age assessment. The participants' task was to classify each image into one of seven age categories (spanning two years).

The results showed that the tested algorithms were challenged by known sex-specific growth patterns. For both sexes, error increased as individuals approached adulthood. Interestingly, NNs and SVM trained on feature points yielded comparable results, with a mean absolute error of less than 1.5 years (the maximum up to 7 years). As expected, the best performance rates were acquired for the pre-trained CNNs. However, for females, age less than 14 years was consistently overestimated, while for older girls, it was underestimated. Conversely, for males, ages 14-15 were identified as the breakpoint beyond which mean absolute error begins to significantly deteriorate. Overestimation in males was evident only at early ages. When confronted with estimates from humans, the automated algorithms produced better results than those by participants, but overall, the estimates were less consistent.

The machine learning approaches have proven that they can be very efficient when trained on a large training dataset. Nevertheless, there are several other issues of computational, technical, and biological nature that need to be addressed in order to acquire truly reliable automated age assessment methods. The purpose of this presentation is to present the issues to the forensic community.

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### Age Assessment, Subadult, Forensic Image Analysis

## C5 Methods for Evaluating and Optimizing Automated Detection/Classification Systems in the Forensic Environment

Jeff Smith, MS\*, MITRE, McLean, VA

**Learning Overview:** After attending this presentation, attendees will better understand the foundational principles underlying detection/classification systems used in forensics, such as biometric recognition and multimedia manipulation detection, considerations for their operational use, and novel techniques for implementing these systems in a forensic environment.

**Impact Statement:** This presentation will impact the forensic science community by increasing baseline knowledge in the operating principles behind automated systems and by proposing best practices and new methods for their implementation in a forensic environment.

It is necessary to implement automated systems in forensics and intelligence in application areas such as biometric recognition and multimedia manipulation detection. These analytics leverage machine learning techniques that output a confidence value or posterior probability of classification given the evidence input.<sup>1</sup> This probability is typically a value between 0 and 1. A threshold, set to 0.5, would assign samples of outputs larger or equal 0.5 to the positive class, and the rest to the negative class.<sup>2</sup> While this arbitrary threshold can work well in many cases, as will be shown in this presentation, an optimal threshold can be found by evaluating performance against known data.

The results of these systems when evaluated against known data can be summarized with a Confusion Matrix (example in Figure 1) of True Positives (TP), False Positives (FP), True Negatives (TN), and False Negatives (FN).

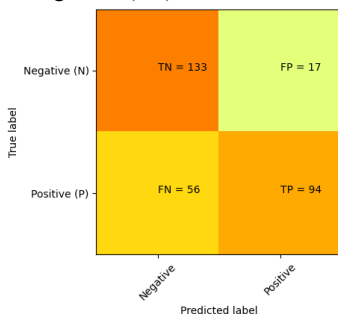


Figure 1: Example Confusion Matrix at an arbitrary threshold shows count of False Positives, False Negatives, True Positives, and True Negatives.

The most common evaluation technique is the area under the Receiver Operating Characteristic (ROC) curve in order to establish overall system performance. The ROC curve (example seen below in Figure 2) is a plot of the system's True Positive Rate (TPR):

$$TPR = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}}$$

over its False Positive Rate (FPR):

$$FPR = \frac{\text{false positives}}{\text{false positives} + \text{true negatives}}$$

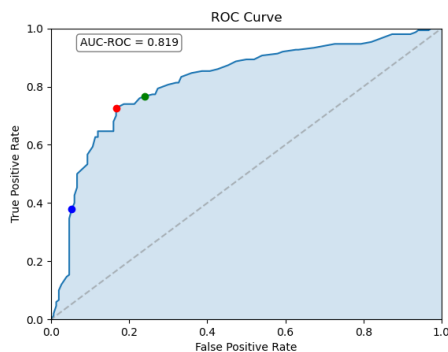


Figure 2: Example ROC curve with AUC, shaded blue, of 0.819. The point where False Positive Rate = 0.05 is labeled with a blue dot, the red dot where MCC is maximum, and the green dot shows TPR and FPR with the threshold at 0.5.

The utility of this plot comes from observing the Area Under the Curve (AUC). This is the definite integral of the ROC curve across all thresholds; a value of 0.819 in example Figure 2. An AUC of 1 would be perfect performance—no false positives, no false negatives. An AUC of 0.5, the dotted line where  $x=y$ , is performance that is no better than chance. In reality, no system is expected to perform perfectly, so an acceptable rate of error must be determined and achieved prior to implementation against unknown, or real, data. By comparing the AUC of detection/classification systems, one can determine the best or most appropriate system given the performance on known data. While the area under the ROC curve is useful for evaluation across all thresholds, the task of selecting one operating threshold is still required prior to implementation in a forensic environment.

During this presentation, the range of optimal thresholds where the TPR and FPR counts are maximized will be explored to develop a novel method of evaluating and selecting a forensically sound operating threshold considering the rate of change between critical thresholds (where the Matthews Correlation Coefficient [MCC] is highest and where the False Positive Rate reaches a desired level).<sup>3</sup>

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1. Platt, J. Probabilistic Outputs for Support Vector Machines and Comparisons to Regularized Likelihood Methods (1999). *Advances in Large Margin Classifiers*, Vol.10(3), p.61.
2. *Scikit-Learn User Guide*. [https://scikit-learn.org/stable/user\\_guide.html](https://scikit-learn.org/stable/user_guide.html), retrieved August 15, 2021.
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### Detection, Comparison, Digital

### C6 A Quantitative Analysis of Video Software Frame Extraction Methods for Use in Forensic Science

*Gregory Wales, MSc\*, University of Colorado Denver, National Center for Media Forensics, Denver, CO; Christopher Lemon, BA, University of Colorado Denver, National Center for Media Forensics, Denver, CO; Gregory Savage, BSc, University of Colorado Denver, National Center for Media Forensics, Denver, CO; Stephen Wells, BA, University of Colorado Denver, National Center for Media Forensics, Denver, CO; Jesus Valenzuela, BSc, University of Colorado Denver, National Center for Media Forensics, Denver, CO*

**Learning Overview:** After attending this presentation, attendees will better understand factors affecting frame/image quality when extracting a video frame for forensic use.

**Impact Statement:** This presentation will impact the forensic science community by providing the community with cross hardware and software test results of some commonly used open-source and commercial video software tools (including some video forensic tools) used to extract frames/still images.

The Video/Image Technology and Analysis (VITAL) Subcommittee of the Organization of Scientific Area Committees for Forensic Science published research needs associated with factors affecting image quality when extracting a still from a video that was approved February 26, 2021. VITAL noted images extracted from videos appear to differ in quality when the same video data is viewed on different computer systems using the same extraction technique as well as different methods. VITAL specifically noted research was needed to determine if computer hardware was a factor affecting image quality when extracting images from video.<sup>1</sup>

The fundamental principle of forensic science and in the handling of digital and multimedia evidence is to ensure the integrity of evidence through the collection and analysis stages of an investigation. As noted in the VITAL research needs document, changes to the video frames have been detected during the extraction process to still images or frames. It is important to gain a better understanding of hardware and software influences in the alteration process for further research. This study expanded on the quantitative comparison of frame extraction methods for Motion JPEG video that were unpublished research previously presented in American Academy of Forensic Sciences conference proceedings in 2014.<sup>2</sup>

The study used five different quantitative analysis methods to test the frame extractions using the various software and hardware compared to carved JPEG frames (ground truth) from the Motion JPEG test data set videos. The testing hardware included multiple operating systems and multiple hardware manufacturers.

This presentation continued the previous research, followed the testing methods in the previous research, and used some of the same quantitative evaluation methods as well as adding additional methods for more granularity of possible changes in the exported frames.<sup>2</sup> The findings address some specific VITAL research interests and revealed trends in frame changes in exported video files. In addition, the study was able to successfully export frames from the same test videos using different test hardware repeatedly without alteration of the exported frames.

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#### Multimedia Forensics, Forensic Video, Image Analysis

### **C7 Can WhatsApp Transmitted Audio Recordings Be Authenticated?**

*Gretchel Lomboy, MSc\*, Seattle Police Department, Seattle, WA; Cole Whitecotton, MSc, University of Colorado Denver, Denver, CO; James Zjalic, MSc, Verden Forensics, Edgbaston, Birmingham, United Kingdom*

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**WITHDRAWN**

### C8 The Use of Electric Network Frequency Presence Due to Rolling Shutter Effect in Video Material for Time Estimation

*Zeno Geradts, PhD\*, Netherlands Forensics Institute, The Hague, South Holland, Netherlands and University of Amsterdam, Amsterdam, North Holland, Netherlands; Guus Frijters, MSc, Netherlands Forensics Institute, The Hague, South Holland, Netherlands*

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**Learning Overview:** After attending this presentation, attendees will better understand that based on the rolling shutter effect of a Complementary Metal-Oxide Semiconductor (CMOS) in combination with the Electric Network Frequency (ENF), it is possible to determine the time when a recording was made.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of the validation of a new method for determining the time of recording ENF based on the rolling shutter effect in CMOS sensors.

Within the forensic field, video evidence becomes more and more relevant due to the wide availability of recording devices everyone carries with them in the form of a smart phone. Different types of crimes are filmed with a variety of camera devices, such as mobile phones. Within video forensics, it is of great importance to verify the authenticity of the videos made with such devices.

Next to the authenticity, the time of recording is important. The time of the video can give a large amount of information, such as proof of someone's whereabouts at a certain point in time. It is crucial that this information is correct and has not been tampered with. A method of time verification is using hidden information (such as metadata) present in videos. One of these types of hidden information is the ENF, which is a fluctuating signal within the power grid. This time-unique signal can be used to determine the time if it can be matched with data.

In this research, the possibility of performing an estimation of the time of recording for videos is explored for different light sources in different circumstances using the ENF. This research focuses on videos made with mobile phones. The mobile phone cameras make use of integrated CMOS sensors. The filmed videos are analyzed using software, which uses an ENF database to determine the time of recording of a video made in experimental circumstances.

This research shows that in ideal circumstances, it is possible to determine the time of recording with a video made with a mobile phone. However, it becomes clear that different light sources greatly influence the results. The best results are achieved with halogen and incandescent light sources, both of which also seem promising in less ideal circumstances. LED light sources do work in ideal circumstances; however, they do not show much success in less ideal situations. This research shows there is potential in using ENF to determine a time of recording of a video. It proves currently usable in ideal circumstances with the presence of a clear light source on a white wall. With more research, it may become a feasible method to use in circumstances that are less ideal.

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#### ENF, CMOS, Time Estimation



### C9 A Review of Axon Citizen™ From a Forensic Perspective as a Tool for Law Enforcement Investigations

*Jesus Valenzuela, BSc\*, Seattle Police Department, Seattle, WA; Gretchel Lomboy, MSc, Seattle Police Department, Seattle, WA; Catalin Grigoras, PhD, National Center for Media Forensics, Denver, CO; Jason Latham, BA, National Center for Media Forensics, Denver, CO*

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**Learning Overview:** Attendees of this presentation will gain insight on Axon Citizen™ public portal features as an asset to law enforcement agencies and be able to understand the known limitations of the software as well as the challenges that software developers face with changing operating systems and third-party updates. This presentation will inform members of the digital multimedia scientific community of the pros and cons of implementing this feature as an investigative tool as well as a discussion on the level of forensic preparedness an organization should consider when exploring this type of digital multimedia evidence collection. After attending this presentation, attendees will better understand the benefits and limitations of using the Axon Citizen™ software as an investigative and digital multimedia collection tool for law enforcement organizations across the nation.

**Impact Statement:** This presentation will provide awareness to a new technology developed for the collection of digital and multimedia evidence and the impetus for the Seattle Police Department (SPD) to use the Axon Citizen™ feature as well as sharing the experimental design, validation results, and insights from a forensic perspective about the emerging technology.

In 2020, the city of Seattle, WA, experienced ongoing protests over the murder of George Floyd. These demonstrations devolved into violent riots that resulted in widespread city property damage and the looting of many local businesses. The SPD was overwhelmed with attempting to identify the instigators and the suspects involved with the destruction of city property, which included several police vehicle units. SPD made the decision to open an evidence submission portal to solicit digital multimedia from the public using a trusted third-party vendor: Axon Enterprise, Inc. The Axon Citizen™ for Communities feature allowed SPD to create a URL and share the submission link through social media. The agency collected thousands of images and videos submitted by the public.

Since then, the SPD has been continuously working on expanding its use of Axon Citizen™ and is currently leveraging the mobile app version that allows officers and detectives to send individual digital evidence upload links to members of the public via Short Message Service (SMS) text or email. The testing and evaluation of Axon Citizen™ for Officers mobile application feature included validation tests of the Axon file transfer protocol performed by SPD in partnership with the University of Colorado National Center for Media Forensics. The purpose of the software test was to verify accurate acquisition and submission of digital multimedia to the Axon Evidence.com platform without alterations.

To further examine the capabilities of Axon Citizen™, a known dataset was created to compare the source files against the files uploaded to and received from the portal. The data analysis was performed between the original source file and the file received on the department's end to gain insights on data integrity, provenance, and authenticity. The mobile features were tested on both Apple® and Android® systems.

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#### Axon, File Transfer, Digital Evidence

## C10 Constructing Digital Video Recorder (DVR) Datasets for Multimedia Forensics Validation

Rayna Mock, BS\*, Marshall University, Huntington, WV; Josh Brunty, MS, Marshall University, Huntington, WV

**Learning Overview:** Attendees of this presentation will understand the importance of validation in digital and multimedia forensics, and how test images for validation are lacking in the area and need to be created, especially for DVRs.

**Impact Statement:** Validation plays a vital role in the field of forensics. International Organization for Standardization (ISO) 17020/17025 require that forensic laboratory disciplines validate instruments to achieve consistent and reproducible results.<sup>1,2</sup> In addition, organizations such as the Scientific Working Group on Digital Evidence (SWGDE), National Institute of Standards and Technology Organization of Scientific Area Committees (NIST OSAC), and American Society for Testing and Materials (ASTM) have all published best practice guides on the validation of digital and multimedia forensics tools. Validation not only ensures that software is working as intended, but also provides reliability and reproducibility in court.<sup>1</sup> While digital forensics has developed formalized validation processes, the subdiscipline of multimedia forensics is lacking in that area. Furthermore, multimedia forensics lacks a formal regulating body, has limited test images to use for validation, and has restricted access to answer keys for many test images that are publicly available.<sup>3</sup>

Test images are key products needed for validation and are created by both formal entities and various individuals for examiners to use when testing a new software or forensic tool. Formal entities such as NIST's Computer Forensics Reference Datasets (CFREDS), The Computer Forensic Tool Testing Project (CFTT), and Digital Corpora host various iOS®, Android®, Universal Serial Bus (USB), and computer-based images available for public download, however, very little exists for multimedia forensics tools. The only available images that exist come from the specific tool vendors themselves, such as DVR Examiner.<sup>4</sup> Lack of access to multimedia forensic test images results in laboratories creating their own, which increases backlog, or requires them to buy from a specific vendor. For example, a request was made by Video/Imaging Technology and Analysis (VITAL) to OSAC, asking that a test image repository be created, since this is lacking in the field.<sup>5</sup>

This research focuses on creating a vendor-neutral multimedia forensic DVR test image that can be publicly available for forensic examiners. A 24-hour loop was recorded on a HeimVision DVR, then forensically imaged in three different formats: E01, raw, and Advanced Forensic Format (AFF). Based off SWGDE's three types of testing, a comparison test was completed by running the information across multiple multimedia forensic software tools.<sup>6</sup> The information gathered from each software tool was noted and compared to one another to determine the accuracy of data produced. The purpose of the project is to create a publicly available and vendor-neutral forensic test image that digital forensic laboratories and examiners can use to validate multimedia forensic tools.

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### Datasets, DVR, Multimedia Forensics

## C11 Likelihood Ratios for Categorical Evidence with Applications in Digital Evidence

Rachel Longjohn, MS\*, University of California-Irvine, Irvine, CA; Padhraic Smyth, PhD, University of California-Irvine, Irvine, CA; Hal Stern, PhD, University of California-Irvine, Irvine, CA

**Learning Overview:** The goal of this presentation is to introduce a likelihood ratio approach for the statistical analysis of cases where the evidence can be broadly described as counts for different categories of interest. In particular, this presentation will focus on applications in digital forensics involving counts of different types of user-generated events on a digital device.

**Impact Statement:** This presentation will introduce to the forensic science community a statistical method for quantifying the strength of categorical evidence in the form of count data and demonstrate how this approach could be used to support digital forensics investigations.

With the widespread use of smart phones and other devices, there exists a growing need for quantitative methods of analyzing digital evidence. Counts of user-generated activities are a common form of data that occurs in digital evidence (e.g., counts of websites browsed, texts/calls/emails sent, apps used, or locations visited). A common defense in cases involving digital forensics is that the recovered device was stolen or otherwise not in the possession of the suspect during the period of criminal activity. However, suppose that investigators can extract from the device historical counts of its user-generated activities, both (1) for the time period the suspect claims to have had the device, and (2) during the time period of the crime. Using this data, one would like to be able to answer questions such as how likely it is that these kinds of activities would be observed if the suspect actually had possession of their device during the period of criminal activity. Being able to answer such questions through robust, quantitative methods has the potential to greatly support investigative efforts in digital forensics.

In this study, a statistical modeling framework is introduced to address such questions when counts of user-generated activities on a digital device are available as evidence. The count data can be split into two time periods, one during which the suspect is known to have had the device and one in which the owner is unknown. The problem can then be formulated using a same-source versus different-source hypothesis framework. Under the same-source hypothesis, it is assumed that the activity counts in both periods were generated by the suspect; while under the different-source hypothesis, it is assumed that the suspect did not generate the counts during the unknown time.

The proposed model utilizes simple, parametric distributional assumptions that have been used across many other non-forensic applications in Bayesian statistics.<sup>1,2</sup> These parametric assumptions lead to a straightforward formula for the likelihood ratio, which measures the relative probability of obtaining the evidence under the same-source and different-source hypotheses and is a widely accepted way to quantify the strength of evidence in forensics.<sup>3,4</sup>

The potential efficacy of the model is demonstrated through an analysis of its theoretical properties and its performance in experiments on real-world datasets involving email communications and device location pings. While the presented framework is motivated by applications in digital forensics, the probability model is intuitive and flexible enough to support its application to a broad variety of forensic evidence types.

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### Likelihood Ratio, Statistical Analysis, Digital Evidence

### C12 Skills for Success: Advancing Pattern of Life Analysis in Cyber Forensics Using a Multidimensional Approach

Mohammad Meraj Mirza, MS\*, Purdue University, West Lafayette, IN and Taif University, Taif, Makkah, Saudi Arabia; Umit Karabiyik, PhD, Purdue University, West Lafayette, IN

**Learning Overview:** After attending this presentation, attendees will better understand the required skills needed in performing Pattern of Life (PoL) analysis for a user using a smart phone such as an iPhone® linked with other smart devices (e.g., Apple® smart watch). From there, this presentation will demonstrate advanced analytical skills using a multidimensional intelligence approach, which aids in investigating and conducting cyber forensics investigations when the required evidence/data are available.

**Impact Statement:** This presentation will impact the forensic science community by providing insights into a set of skills and advanced analysis techniques needed when investigating iPhone® devices containing geolocation data and vital human data such as heart rate gathered from smart watches. In addition, this presentation will show how to transform information and the available data into knowledge to aid investigations.

Everyone nowadays relies on their smart phones to keep up with the digital world and stay in touch with other sophisticated and smart objects such as Internet of Things (IoT) devices in their everyday lives. One example of these advanced devices is the Apple® Watch®, a computationally powerful device despite its diminutive size. It packs a punch when it comes to functionality, and with each new model, it gets more intelligent and versatile. Depending on which model/series, it can do everything from viewing incoming notifications, tracking activities and location, checking and monitoring heart rate, measuring blood oxygen level, and even taking an Electrocardiogram (ECG), which is only available on newer models.<sup>1</sup> Aside from that, as smart watches and other smart objects become more capable; smart phones that are connected to these smart devices receive an enormous amount of data. Therefore, these data can help highlight Personal Identifiable Information (PII), which is considered valuable to digital forensics investigations.<sup>2</sup> Yet, the PII needs to be complemented with different analytical skills and techniques to unlock their full potential.

The PoL is an analysis that evaluates behaviors, and this study focuses on the temporal and spatial dimensions.<sup>3</sup> As a result, this type of analysis can assist in transforming information into knowledge and a storyboard that can explain the user's actions and activities that are not visible in standard analytical approaches (e.g., timeline analysis). It is one type of intelligence technique that aims to aid investigation to uncover the story and quickly identify critical actions considered valuable in investigations. Moreover, PoL analysis can help increase the value of information by giving it a meaningful context and creating a rationale to aid in answering the 6 Ws (i.e., who, what, when, where, why, and how).

In addition, this research uses PoL as a supportive analysis approach complemented by other types of intelligence domains (e.g., activity-based intelligence and location intelligence) to improve geo-mapping of collected information gathered from different systems and applications artifacts to help build a mapped storyboard. This digital storyboard is intended to aid in creating a representational geo-map that provides a significant contextual meaning of the collected evidence. As a result, digital forensic analysts and practitioners may construct models of scenarios and highlight critical events that can help predict what has possibly happened by using this multidimensional approach.

This presentation will demonstrate the skills and techniques discussed using a digital forensic case study, illustrating the aforementioned analytical techniques to highlight the benefits and challenges of the multidimensional approach and the intelligence-related knowledge demonstrated generated by linking what is known with what could enhance and improve digital forensic practitioners' decision-making processes.

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#### Cyber Forensics, Multidimensional Intelligence Approach, Pattern of Life Analysis

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### C13 Virtualization, Self-Hosting, and File/Storage Systems—Recent Technological Advances and the Search for Digital Evidence

*Jason Paroff, JD\*, Epiq, New York, NY*

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**Learning Overview:** Technology is ever-changing, and today’s technology requires an investigator or attorney to understand where essential digital evidence may be located. This presentation will expose attendees to open source and or free-to-use software that allows for easy virtualization of computers and the creation of networked attached storage systems with different types of drive arrays and structures. Items attendees will be exposed to will include: virtualization using Proxmox; different storage types within Proxmox; Virtual Machines and Linux Containers (LXC) within Proxmox; Open Media Vault Network Attached Storage (NAS); physical drives on Proxmox “passed through” to Open Media Vault; different storage types (including Redundant Array of Inexpensive Disks [RAID] arrays) supported by Open Media Vault; and, time permitting, the Union File System (UnionFS) and what it does.

**Impact Statement:** Yesterday’s examiner was 80% detective and 20% IT person. Today’s examiner probably needs to be the reverse—80% IT person and 20% detective. With new advents in technology, yesterday’s investigator, and the litigators they gather evidence for, has had to gain more and more IT skills to remain in the hunt for today’s evidence. Today’s data isn’t where it used to be, and the search for evidence may involve machines that don’t even physically exist (e.g., virtual machines), so today’s investigator needs to learn about these technologies so he/she can locate relevant evidence that simply would not have existed in this form only a few short years ago.

The concept of virtualization—the creating of entire computers and their storage disks within the physical hardware of a larger and more powerful computer—complicates matters today. In essence, what once was a physical laptop, desktop, or server can now be mimicked by software, sharing the hardware of a larger computer in the process. In this way, one physical machine can contain many virtual machines, so the number of “computers” a person can have and control can be significantly greater than it used to be. Through virtualization, you can now click on an icon and spin up an entire virtual computer that acts in every way like its bare metal counterpart, but you can now locate that computer in any part of the world. Finished using one of those machines for the day? Simply shutdown Windows® (or Mac® or Linux®) just like any other computer and the computer disappears from the network and returns to nothing more than a set of software instructions concerning how it uses the larger machine once you decide to use it again. Want to destroy all of the data/evidence on one of those virtual machines? Simply delete it, and it is gone forever, but don’t worry, you can create a new one in about five minutes if you want to.

Now that broadband is readily accessible to many consumers across the globe, the ability to self-host applications is likewise growing quickly. Through self-hosting, it is now possible for average consumers to run applications and services like a website, blog, media server, DropBox alternative, Google® Drive, and OneDrive® alternatives and make all of their files accessible to themselves from anywhere in the world.

Today’s attorneys, investigators, and analysts need to consider virtualized computers as potential sources of relevant data, and due to the ease with which they can be switched off or deleted, may need to move quickly to preserve that data if it may be relevant to an investigation or litigation. This presentation will expose attendees to many of these concepts and options so they can be better informed as to where to look for potentially relevant and important evidence during their next investigation or litigation.

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#### Virtualization, Self-Hosting, Data Storage

### C14 Keeping Forensic Tools Sharp: A Case Study of Updating Bulk\_Extractor 1.6 to 2.0

*Simson Garfinkel, PhD\*, George Washington University, Washington, DC*

**Learning Overview:** After attending this presentation, attendees will understand the need to keep digital forensics tools up-to-date, the resources available for doing so, and the amount of effort required. In particular, attendees will understand developments in the C++ and Python® programming languages over the past decade, and the opportunities to combine digital forensics reference data sets with modern software development practices to improve reliability and reproducibility of digital forensics tools while decreasing error rates.

**Impact Statement:** This presentation will motivate those in the digital forensics community to re-evaluate tools that have long been in use but have not been subjected to rigorous software development practices.

Digital forensics practitioners make extensive use of both proprietary and open-source software for extracting digital evidence from devices under study, for transforming evidence into a form that is readily understandable by examiners, and for organizing and correlating the vast quantities of digital evidence into a form that can be summarized and presented to investigators and juries. Like all software, digital forensics software must be maintained after it is created or it will eventually cease to function. Unlike traditional forensics, where reagents expire unless they are refreshed, forensic software aging happens because of changes in the underlying operating system on which the software executes and because the data under analysis increasingly comes from applications and systems that did not exist when the analysis software was written.

Maintaining digital forensics software is especially expensive, because forensics software must be able to analyze not just data created by all systems being sold in the market today, but data created by any system to which the subject may have had access.<sup>1</sup> Fortunately, advances in software development over the past decade have the potential to significantly decrease the cost, keeping digital forensics tools up-to-date while improving their overall quality. This includes the increased sophistication of underlying programming languages such as C++17 and Python® 3.9, the widespread availability of Continuous Testing and Continuous Integration platforms, wider use of unit testing and code coverage tools, and the increased sophistication of fuzzing technology.<sup>2</sup> Separately, the development of reliable digital forensics tools is aided by the increasing availability of complex reference data sets that can be used for both tool testing and training.<sup>3</sup> Nevertheless, migrating digital forensics software developed over the past two decades to use these new technologies is a significant undertaking.

Presented here are the results of migrating the bulk\_extractor forensic tool from the decades old C++98 standard, to the modern C++17 standard and documenting the increase in both performance and reliability that resulted from the move to modern software development techniques.<sup>4</sup>

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2. M. Shahin; M. Ali Babar; L. Zhu. Continuous Integration, Delivery and Deployment: A Systematic Review on Approaches, Tools, Challenges and Practices. *IEEE Access*, vol. 5, pp. 3909-3943, 2017, doi: 10.1109/ACCESS.2017.2685629.
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**Open-Source Digital Forensics Tools, Bulk\_Extractor, Digital Corporation**

### C15 A Case Study of Virtual Programmable Logic Controllers (PLC) Forensic Framework on Investigating Control Logic Attacks in a Belt Conveyor System

*Syed Qasim, BS\*, Virginia Commonwealth University, Richmond, VA; Irfan Ahmed, PhD, Virginia Commonwealth University, Richmond, VA*

**Learning Overview:** After attending this presentation, attendees will understand control logic attacks on a belt conveyor system and their network forensic investigation using a virtual PLC forensic framework. This presentation will cover a general architecture of PLCs, control logic attacks, and the challenges to investigate them in an industrial control system environment. It will further discuss a scalable virtual PLC forensic framework to recover and analyze a (malicious) control logic. The framework is tested on a heterogeneous Industrial Control System (ICS) environment for a belt conveyor system containing PLCs of different vendors, including Allen-Bradley MicroLogix™ 1400, 1100, and Micro 820 and 850, AutomationDirect CLICK, and Schneider Electric Modicon M221.

**Impact Statement:** This presentation will impact the forensic community by discussing an automated, scalable virtual PLC forensic framework to investigate control logic attacks on a belt conveyor system.

In ICS, PLCs use control logic to control physical, industrial, and infrastructure processes, such as nuclear plants. Attackers target control logic in a PLC to sabotage a physical process. For instance, Stuxnet demonstrates a control logic attack by infecting the control logic of a Siemens S7-300 PLC to manipulate the motor speed of centrifuges periodically to damage them permanently. Often, the control logic attacks compromise the control center and further transfer a (malicious) control logic over the network from the control center to a target PLC remotely. Suppose the network traffic of the control center is captured. In that case, it will contain the attacker's malicious control logic (forensic artifact) in the network stream. The challenge for a forensic investigator is to identify and recover the binary control logic from the network traffic streams and further transform it into a high-level control language such as ladder logic.

Recently, this study has developed a virtual PLC forensics framework to recover control logic from a network traffic dump.<sup>1</sup> This presentation will present a case study of the framework on a belt conveyor system. The framework consists of a virtual PLC that engages with the engineering software using a previously captured network traffic (forensic artifact). Mainly, it utilizes a commonly available upload function in engineering software to retrieve the control logic from a PLC.

The case study was performed on a working model of an industrial conveyor belt used to sort different types of objects. The model consists of capacitive and inductive sensors to differentiate between metals and plastic objects. When a metal object on the conveyor belt passes under the inductive sensor, the sensor senses it and sends a signal to the PLC. After processing the signal, the PLC opens the air solenoid valve to push the object out of the conveyor belt into the sorting storage. Similarly, the capacitive sensor sorts the non-metal objects.

Two attack scenarios were created for the conveyor belt. In the first scenario, the attacker penetrates the ICS network and conducts reconnaissance. The attacker steals the control logic running on the PLC to gather information about the industrial environment. This is done using the upload function of a PLC: the attacker sends the upload request to the PLC, and the PLC, in return, sends the control logic to the attacker in a series of messages. After successful reconnaissance, in the second scenario, the attacker develops a malicious control logic targeting different components, such as input, output, timer statement, etc., and downloads it to the PLC to disrupt the physical operation of the conveyor belt. The attacker inverts the output to air solenoid valves (actuator), which disrupts the sorting process.

To replicate the heterogeneous industrial environment, the belt conveyor system is connected with different PLCs controlling different belt sections. The case study utilizes Modicon M221 and MicroLogix™ 1400 PLCs of leading ICS vendors (i.e., Allen-Bradley and Schneider Electric [a 30% share of the PLC market]).<sup>2,3</sup>

#### Reference(s):

1. S. Qasim; J. Smith; I. Ahmed. Control Logic Forensics Framework using Built-in Decompiler of Engineering Software in Industrial Control Systems. *Forensic Science International: Digital Investigation*, Volume 33, Supplement, 2020.
2. *Market Share Of Different PLCs*. <https://ipcsautomation.com/blog-post/market-share-of-different-plcs/>.
3. *Top 20 programmable logic controller manufacturers*. <https://roboticsandautomationnews.com/2020/07/15/top-20-programmable-logic-controller-manufacturers/33153/>.

#### SCADA Forensics, Network Forensics, Ladder Logic

### C16 Exploiting the Post-Attendee URL Feature in Zoom Webinar to Distribute Malware: Implications for Digital Forensics

*Austin Cauley, BS\*, University of Central Oklahoma, Edmond, OK; Mark McCoy, EdD, University of Central Oklahoma, Edmond, OK*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the methods used by malicious actors to spread malware via Zoom® Webinar and the types of digital artifacts left by these attacks.

**Impact Statement:** This presentation will impact the digital forensics community by illustrating how the post-attendee URL feature within Zoom® Webinar can be exploited by sending webinar attendees to a compromised website.

The ability to force a Zoom® Webinar attendee to visit a compromised website without permission of the attendee is a security flaw that could be leveraged by someone hosting a Zoom® Webinar. These compromised websites, when accessed, can contain malicious code that negatively impacts the attendee.

With the current pandemic causing many businesses to operate virtually to maintain public health guidelines, the video conferencing application known as Zoom® has skyrocketed in popularity. Unfortunately, due to Zoom's® rapid rise in popularity, it has also become infamous for its security vulnerabilities, such as Zoom® bombings that involve projecting illicit material in a Zoom® meeting.<sup>1</sup> As a result, both security researchers and bad actors alike have entered an arms race to see who can discover vulnerabilities within Zoom®. While different methods have been discovered by both sides, there is one feature that is often overlooked: the post-attendee URL within Zoom® Webinar.

This study tested the post-attendee URL feature within the Zoom® desktop application by creating a purposefully compromised website that contained a keylogger that sent data to a third-party web server. Once created, a Zoom® Webinar event was created with the post-attendee URL set to the URL of the purposefully compromised website.

Four computers with new installations of Windows® 10 version 2004 were used as participants in the Zoom® Webinar event with each assigned one of the roles as defined by Zoom®: host, co-host, panelist, and attendee.<sup>2</sup> Each computer used the Zoom® desktop application version 5.7.5 and a baseline image was completed.

Each computer, aside from the computer designated as the host for the webinar, joined the webinar in one of two methods in order to determine what conditions cause the post-attendee URL to trigger. First, each desktop joined the webinar by launching the Zoom® desktop application and manually entering the webinar ID and passcode. After waiting for five minutes once the co-host, panelist, and attendee joined the webinar, it was determined that this joining method does not trigger the post-attendee URL to launch. However, when the co-host and attendee desktops joined via the email link that was sent to their email account after registering for the webinar, the post-attendee URL was successfully launched five minutes after joining the webinar. The keylogger that was embedded into the compromised website functioned as intended and successfully transmitted the user keystrokes on the compromised website to the third-party webserver. After the post-attendee URL was successfully opened on the attendee machines, FTK® Imager was used to capture RAM, then all the computers were imaged again. Once imaged, the Windows® Registry and web browser history were analyzed to discover potential digital artifacts that were created due to the malicious webinar.

The goal of this research is to not only determine the possibility of distributing malware to unsuspecting users, but to also discover and analyze the types of digital artifacts that are left behind from this type of attack. This will assist digital forensic examiners in the future by providing the information they need in order to create a more comprehensive timeline of events for cases that involve this type of attack.

#### Reference(s):

1. Mahr, Andrew; Cichon, Meghan; Mateo, Sophia; Grajeda, Cinthya; Baggili, Ibrahim. Zooming into the pandemic! A forensic analysis of the Zoom Application. *Forensic Science International: Digital Investigation*. 2021; 36: 1 - 12.
2. Roles in a webinar. *Zoom Help Center*. n.d. Retrieved April 19, 2021.

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Digital and Multimedia Forensics, Malware, Zoom®



### C17 Revealing the Invisible: Investigating Evidence of Automated and Behind-the-Scenes Computer Activity

Josiah Dykstra, PhD\*, National Security Agency, Fort George G. Meade, MD

**Learning Overview:** After attending this presentation, attendees will be able to describe the forensic implications of automated and behind-the-scenes cybersecurity now in practice on modern computers and networks and effective techniques to detect and explain such activity in forensic timelines.

**Impact Statement:** This presentation will impact the forensic science community by illuminating the forensic evidence of increasingly automated cybersecurity mechanisms that take place without overt end-user interaction and considerations for legal prosecution.

The field of usable security is a domain of human-computer interaction that attempts to create and evaluate cybersecurity solutions through the lens of user experience. Many users continue to struggle using cybersecurity tools, making decisions, or matching their intentions for security with the reality of their actions.<sup>1</sup> Expanding usability further, this study describes invisible security as an approach where cyber defenses occur automatically and transparently without end-user intervention. For example, automatic software updates and protective Domain Name System (DNS) are examples of this approach.<sup>2</sup> Invisible security helps average users and small businesses who lack the time, expertise, or funding for proper security. While this evolution is valuable to some, usable security impacts digital forensics because users may be unaware if or how their experience is being manipulated to protect them.

Invisible security may pose an impediment to the investigative process by obscuring or manipulating the environment, including the crime scene. For example, protective DNS is a defense against malware whereby a service provider filters domain name lookups. A byproduct is that users or attackers behind protective DNS may be unwittingly redirected or experience an unexpected interruption with internet services. Forensic investigators must understand if and how automatic updates are present and how to identify, preserve, collect, examine, analyze, and present the additional evidence. In some cases, the relevant data exist on other systems that may be controlled by third parties such as internet service providers. Automatic software updates similarly offer the opportunity for software to change with the user's awareness. To forensic investigators, invisible security may introduce new evidence in the timeline of events and introduce uncertainty about the source of observed activity.

This presentation describes common examples of invisible security today, including automatic updates, and changes on the horizon, including invisible user authentication. This presentation presents indicators and recovery techniques to aid forensic investigators in identifying and documenting invisible defenses in the crime scene. Finally, this presentation offers perspectives about the technical and legal implications of automated security to forensic evidence and prosecution. These results may inform future digital forensics of other automated and artificial intelligence systems.<sup>3</sup>

#### Reference(s):

- <sup>1</sup> Dykstra, J.; Spafford, E. The Case for Disappearing Cyber Security. *Communications of the ACM*. 2018, 61(7), 40-42.
- <sup>2</sup> Wash, R.; Rader, E.; Vanica, K.; Rizer, M. Out of the Loop: How Automated Software Updates Cause Unintended Security Consequences. *Symposium on Usable Privacy and Security (SOUPS)*. 2014, Menlo Park, CA, 89-104.
- <sup>3</sup> Baggili, I.; Behzadan, V. Founding The Domain of AI Forensics. *Proceedings of the Workshop on Artificial Intelligence Safety (SafeAI)*. 2020, New York, NY, 31-35.

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Cybersecurity, Digital Forensics, Automation

### C18 The Decontamination of Drug-Contaminated Mobile Devices Using VTO Labs' Decontamination Kits

*Arcelia Cohen\**, Marshall University, Huntington, WV; *Joshua Brunty, MS*, Marshall University, Huntington, WV; *Lauren Richards-Waugh, PhD*, Marshall University, Huntington, WV

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of needing an applicable decontamination protocol to safely handle and analyze drug-contaminated digital evidence.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence that the decontamination protocol established by VTO Labs is a method that can be applied in the field.

There is a problem in digital forensics in which evidence seizure and data extraction cannot be completed due to possible health risks to seizure and examiner personnel. With rising drug use, more drug-contaminated devices are being seized. Previously, these devices were kept in storage because analysts could not safely handle the devices.<sup>1</sup>

Understanding this issue, VTO Labs developed a decontamination protocol that not only protects the forensic analysts but also the personnel collecting the devices. After decontamination, analysts can safely retrieve the vital information kept on the devices.<sup>1</sup> The process is known to clean fentanyl, carfentanil, and methamphetamine residues from the devices.<sup>1</sup> Given the potential contact for additional substances, heroin, cocaine, and cocaine with suspected fentanyl were tested on various devices.<sup>2,3</sup>

Nine different devices were contaminated with a drug sample from a Huntington Police Department case. These samples were scheduled for disposal and had previously been analyzed by the West Virginia State Police Forensic Laboratory. The contaminated devices were swabbed in three different locations using Kimwipes®, and the samples were extracted using methanol and a vortex.<sup>4</sup> After the VTO Labs' decontamination process was completed, the surfaces were swabbed, and the samples extracted in a similar manner. The extracted samples were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), and the data was compared to determine if the decontamination process removed all traces of the controlled substances.

The contaminated samples showed that the different locations provided varying levels of drugs detected. VTO Labs' decontamination process produced a variety of observable damage to each of the devices; however, the decontamination process seemed to be an effective tool that can be utilized in the field.

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- <sup>1.</sup> *Decontamination of Electronic Devices*. vto-labs-training.thinkific.com/courses/decontaminate-electronic-devices (accessed July 16, 2021).
- <sup>2.</sup> Fentanyl. dea.gov/factsheets/fentanyl (accessed July 26, 2021).
- <sup>3.</sup> Park, J.N.; Rashidi, E.; Foti, K.; Zoorob, M.; Sherman, S.; Alexander, G.C. Fentanyl and fentanyl analogs in the illicit stimulant supply: Results from U.S. drug seizure data, 2011-2016. *Drug and Alcohol Dependence* 2021;218.
- <sup>4.</sup> Ciesielski, A.L.; Wagner, J.R.; Alexander-Scott, M.; Snawder, J. An optimized method for sample collection, extraction, and analysis of fentanyl and fentanyl analogs from non-porous surface. *Talanta* 2021 Feb;228.

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#### Decontamination Process, Digital Devices, Cocaine

## C19 Crowdsourcing Forensics: The Digital Artifact Catalog

*Lam Nguyen, MS\*, Department of Defense, DC3, Linthicum, MD; Eoghan Casey, PhD, Department of Defense, DC3, Linthicum, MD*

**Learning Overview:** The aim of this presentation is to propose a framework for modeling digital forensic artifacts in order to overcome challenges associated with collating and curating investigative findings. This presentation will provide a prototype of the proposed model and discuss distribution options. Attendees will understand how artifacts can be structured in a manner that supports crowdsourcing and standardization.

**Impact Statement:** It is infeasible for an individual, or even a single organization, to keep pace with the growing number of applications on a variety of computing devices. As a result, there is an increasing risk that forensic practitioners will miss or misinterpret digital artifacts.

Prior work demonstrated the value and difficulties of designing a repository of digital artifacts.<sup>1</sup> On the one hand, the complexity of an ontology-based approach makes implementation more difficult. On the other hand, the oversimplification of treating digital artifacts as Files with tagging lacks precision and leaves it to individual users to infer what piece of data within the File is relevant and what the data represents. To strike a balance between these two extremes, the Digital Artifact Catalog has been conceived and populated by practitioners based on observations and insights from actual forensic examinations.

The Digital Artifact Catalog provides the forensic community with a robust framework for organizing and sharing knowledge about digital artifacts. Each entry in the Digital Artifact Catalog contains the details necessary to pinpoint the associated unit of interpretable data that is useful for addressing a specific question and cites supporting documentation and/or research results. References to external resources provide additional information for practitioners to learn more about specific item of data. This information is useful for tool development purposes and explaining digital evidence in court.

This initiative reduces wasted time and effort of each individual or organization studying digital artifacts independently, and it allows the relevant scientific community to establish general acceptance for specific digital artifacts. In addition, community curation of knowledge about digital artifacts helps increase thoroughness, consistency, and repeatability of digital forensic results. A longer-term ambition of the Digital Artifact Catalog is to aggregate statistics about digital artifacts to shed light on which are most useful for answering source-level and activity-level questions. More than ever, forensic practitioners need to have intimate knowledge of a wide array of digital devices, data structures, and network inter-operability. The process of interrogating a device for evidential data has become more art than science, making it increasingly hard for automated tools to keep pace. It can take weeks to research, experiment, and develop a novel approach to a single problem.

Yet examiners are expected to recognize the evidentiary significance of every possible data item on a device. They must be able to identify how the data is stored, physically locate it, and have the expertise to extract that data into human readable format. Theoretically, this process must be completed not just for every app, but every permutation of the app, its version, the hardware device version as well as the Operating System that runs it. The data structure of a banking app may be completely different from one version to the next. Or the same version may store data differently on Android® devices versus an Apple®-based iOS® device.

A data artifact, then, is a singular unit of interpretable data that can be extracted from a digital device. To be useful, it must be associated with the device and its specific operating system, the precise program that created the data, the location it was found, and the specific method used to extract the data.

Unfortunately, not all examiners can identify all possible data artifacts and interpret them. This isn't a weakness in the examiner. Digital forensics is unique in that every examination may involve never-before-seen data. It would be virtually impossible for an individual to know how to extract every single piece of data from every device they may encounter. In a world where technology is consumable and devices can be refreshed in less than a year, the problem is seemingly insurmountable. Across the country, digital forensic labs grapple with this issue. The problem is exacerbated within the context of law enforcement where reports are subject to an in-depth examination by prosecutors, defense attorneys, judges, and jury. Because of the complexity of litigation, the method of digital data extraction must be predicated on the principles of forensic science. It must be validated, documented, and determined to be forensically sound, all while still maintaining evidential integrity.

By using a common platform shared across public, private, and academic environments, practitioners can leverage and contribute to a crowdsourced knowledge repository that benefits the entire community. Rather than duplicate effort, participants would pool resources to tackle what would otherwise be an impossible problem for a single lab. The collaborative nature of a crowdsourced project is entirely dependent on the ability to organize and seamlessly share data across multiple users. It must be easy to use, readily available, and provide reciprocal benefit to its users. The proposed standards will allow practitioners to not only share artifacts as they are found, but to share the method used to extract and interpret them. As these artifacts and their respective data extraction methods are shared, a community of users are available to test, validate, and provide widespread acceptance and support.

What would otherwise take days, if not weeks, could be reduced to a simple database lookup. Labs would be able to validate and cross-reference materials produced by practitioners not only in their own lab; but with other labs across the entire forensic community. Smaller labs that do not have the resources to experiment or test methods would have access to a plethora of data that they may not have even realized was available.

### Reference(s):

- <sup>1</sup> Grajeda, C.; Sanchez, L.; Baggili, I.; Clark, D.; Breiting, F. 2018. Experience constructing the artifact genome project (agp): Managing the domain's knowledge one artifact at a time. *Digit. Invest.* 26. <https://doi.org/10.1016/j.diin.2018.04.021>. S47 e S58.

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### Crowdsource, Artifacts, Digital Evidence

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## C20 Standards Development Activities in Digital and Multimedia Forensic Science

*Tracy Walraven, MFS\*, DC Department of Forensic Sciences, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of digital and multimedia forensic science.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to digital and multimedia issues. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to digital and multimedia standards development will be discussed. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC proposed standards; (3) published standards from the American Society for Testing and Materials (ASTM) and other authoritative bodies that have yet to go through the Registry approval process or are currently undergoing the approval process; (4) priorities for new documents or work products, and other highlights; and (5) projects undertaken by working groups within the digital evidence subcommittee, specifically the Quality Study Working Group's project entitled Quality Management in Digital Evidence Examination that interviews various members of the Digital & Multimedia Evidence (DME) and legal community to determine which elements of a quality system are most effective in digital forensics. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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**Digital/Multimedia, Standards, OSAC**

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## C21 Key Takeaways from a National Institute of Standards and Technology (NIST) Digital Forensic Scientific Foundations Study

*James Lyle, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Barbara Guttman, BA, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending this presentation, attendees will be aware of some of the key takeaways from the National Institute of Standards and Technology (NIST) Digital Forensics Foundation Study.

**Impact Statement:** This presentation will impact the forensic community by increasing awareness in the community of available resources used to establish the reliability of digital forensic techniques and tools. This presentation will aid forensic practitioners in developing their own methods and materials for describing the reliability and fitness for purpose of digital forensic tools and techniques used in their investigations or locating materials that can be used to support statements about the reliability of the techniques and tools they have used.

In recent years, several scientific advisory bodies have expressed the need for scientific foundation reviews of forensic disciplines and identified NIST as an appropriate agency for conducting them. The purpose of a scientific foundation review is to document and consolidate information supporting the methods used in forensic analysis and to identify knowledge gaps where they exist. In addition to digital evidence, NIST is conducting scientific foundation reviews in DNA mixtures, bitemarks, and other areas.

One of the first key takeaways is that the details of digital tools and techniques evolves rapidly as the digital technology changes. Other key takeaways include:

- Digital techniques are a vast array of techniques as numerous as all the non-digital investigation techniques.
- The digital landscape evolves rapidly and in unexpected directions.

Validation of a technique is not the same as tool testing. Validation of a technique is demonstration of “fitness for purpose.” For example, testing a hashing tool to demonstrate that the tool computes the hash correctly is not sufficient to show that the right tool has been selected. It must also be shown that the hash algorithm has the desired set of properties such as collision resistance, etc.

Verification of tool implementations needs to be better supported. Only a tiny fraction of available tools receives any independent testing.

Software tools will never be perfect, they will always have flaws and quirks. The investigator needs to be aware of any flaws in the lab’s toolkit so that steps can be taken to mitigate the tool’s shortcomings.

Software tools that have been tested have not exhibited flaws that are likely to change the outcome of an investigation. The usual flaws observed would cause random things to be missed and therefore not considered.

Some identified gaps and needs include:

- There needs to be a central repository of digital artifacts that describes the artifact, the significance of the artifact, and what situations the artifact occurs in. There have been several attempts to create such a repository, but there needs to be more work.
- There is not a consensus in the community that establishes the requirements to demonstrate “fitness for purpose” for all the techniques in use.
- There need to be more validation studies to show “fitness for purpose” and to identify weaknesses in digital methods. There have been a few studies, but the diversity of case work needs to be considered.

In summary, while many digital tools have been shown to be effective and their use has been informed by known limitations, the field needs to evolve to test new tools and techniques more rapidly and comprehensively. As digital continues to evolve to analyze ever more devices, applications, and data collection, this will become even more important.

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### Digital, Verification, Validation

### C22 What Is the Computer Forensic Tools and Techniques (CFTT) Catalog?

*Jenise Reyes-Rodriguez, BS\*, National Institute of Standards and Technology, Gaithersburg, MD; Richard Ayers, MS, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending this presentation, attendees will better understand the Computer Forensic Tools and Techniques catalog: what is it used for, how the forensic community would benefit from it, how to access it, and what it looks like.

**Impact Statement:** The content of this presentation will impact the forensic community by providing an overview of a Forensic Tools and Techniques catalog that the whole community has access to and can be beneficial not only to forensic examiners but to all forensic tool users in general.

As technology changes rapidly, the Computer Forensics Tool Testing (CFTT) program updates its documents to keep up and offer the forensic community current and relevant information. The Tools and Techniques catalog is an exception to the rule. In this case, instead of CFTT populating it and keeping it updated, it is populated and updated by the forensic tool vendors. The CFTT project maintains the website and makes sure the information is accurate and up to date.

The Computer Forensic Tools and Techniques catalog provides the ability to search by technical parameters based on specific digital forensic tool functions, such as disk imaging or deleted file recovery. All the information in the catalog is provided by the developer. All commercial or non-commercial products within the catalog are for information only and do not imply that the product has been tested. The catalog is still growing, and there is work on adding some forensic techniques soon.

In addition to the catalog offering a list of tools that the user can search based on the functionality of interest, the forensic community could also suggest changes to the catalog. Suggestions to changes may include changes to the catalog's taxonomy, adding search features, or suggesting new functionalities that may not be included within the catalog. This allows the forensic community to collaborate and engage with the catalog.

The primary goal of the Tool catalog is to provide a simple searchable catalog of forensic tools and techniques. This enables practitioners to find tools and techniques that meet their specific technical needs. A secondary goal of the Tool catalog is to provide a picture of the digital forensics tool landscape, showing where there are gaps (i.e., functions for which there are no tools or techniques).

Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement by the author or the author's employer, nor does it imply that the products are necessarily the best available for the purpose.

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#### Computer Forensics, Tools and Techniques Catalog, Forensic Tools

### C23 A Forensic Analysis of Microsoft® Teams

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**Learning Overview:** After attending this presentation, attendees will understand what artifacts the Microsoft® Teams client stores on iOS®, Android®, and Windows® 10, as well as how they are stored.

**Impact Statement:** Microsoft® Teams is a largely undocumented platform from a forensic standpoint. This presentation will provide important information about how the platform stores user data across three different devices. How platforms handle data is useful not only to the forensic science community, but also the end users who use the platforms.

Digital forensic investigators have a duty to understand the relevant components of the cases they work. However, with the constant evolution of technologies, and the release of new platforms and programs, it is impossible for an investigator to be familiar with every application they encounter. It can also be difficult to know how forensic tools handle certain applications. This is why forensic researchers study and document new and emerging technologies, platforms, and applications, so that investigators have resources to utilize whenever they encounter an unfamiliar element in a case.

In 2017, Microsoft® released a new communication platform, Microsoft® Teams.<sup>1</sup> As of April 2021, Microsoft® Teams had 145 million daily active users—nearly double the number of daily users, at the same time, one year earlier.<sup>2,3</sup> This rapid growth is attributed in part to the COVID-19 pandemic, as nearly all social, educational, and professional lives moved online.<sup>3</sup> Given the size of its user base, it seems likely that forensic investigators will encounter cases in which Microsoft® Teams is a relevant component. However, due to the application’s relatively young age, limited forensic research exists on Microsoft® Teams.

To address this gap, an analysis of data stored at rest by Microsoft® Teams was conducted, both on the Windows® 10 operating system as well as on mobile operating systems (iOS® and Android®). Basic functionality, such as messaging, sharing files, participating in video conferences, and other functionalities that Teams provides were performed in an isolated testing environment. The test devices were analyzed using both forensic tools’ automated artifact finding capabilities, and non-automated investigation by investigating file system structures in details. Specifically, Cellebrite Universal Forensic Extraction Device (UFED) for the mobile devices, and Magnet AXIOM for the Windows® device were used. Manual or non-automated investigation recovered, at least partially, 77.6% of all artifacts across all three devices. For both mobile devices, at least 90% of populated artifacts were recovered with non-automated investigation, while 41.3% were discovered for the Windows® device. In this study, the forensic tools used automatically recovered and identified 13.7% of all artifacts across all three devices, recovering no more than 20% of populated artifacts for any device. These discovered artifacts, and the full results of this study, are documented in the hopes of aiding future digital forensic investigations involving Microsoft® Teams.

#### Reference(s):

1. Koenigsbauer, K. (2017, Mar). *Microsoft teams rolls out to office 365 customers worldwide*. Retrieved from <https://www.microsoft.com/en-us/microsoft-365/blog/2017/03/14/microsoft-teams-rolls-out-tooffice-365-customers-worldwide/>.
2. Wright, T. (2021, Apr). *Teams hits 145 million daily active users*. Retrieved from <https://www.uctoday.com/collaboration/teams-hits-145-million-daily-active-users/>.
3. Zaveri, P. (2020, Apr). *Microsoft teams now has 75 million daily active users, adding 31 million in just over a month*. Retrieved from <https://www.businessinsider.com/microsoft-teams-hits-75-million-dailyactive-users-2020-4>.

#### Microsoft® Teams, Mobile Forensics, Tools

### C24 The Correlation of Garbage Collection and Object Recovery in Userland Forensic Analysis

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**Learning Overview:** After attending this presentation, attendees will understand how Garbage Collection (GC) can affect object recovery in userland forensic analysis. The research highlights the impact of each type of GC on object recovery and how effectively the types of GC analyzed can impact object recovery for solving cyber cases.

**Impact Statement:** This presentation will impact the forensic community by illustrating how the type of GC that occurred before or during memory acquisition impacts the kind of evidence that may likely be recovered during a memory forensics investigation.

Userland memory forensics is a forensic technique to retrieve artifacts that provide sufficient information to recover application functionality by outlining the actions without prior knowledge of application logic. One of the major steps in most of the userland forensic analysis is process memory acquisition, which may be a dump acquisition instance when the runtime triggered a GC to occur, leading to the loss of few objects that may or may not serve as a piece of crucial evidence for investigation. Android® GC can impact the availability of evidence, as it is an automatic process that removes unused objects from memory. This research will demonstrate how different types of GC introduced by Android® Runtime(ART) can cause a change in objects recovered by forensic tools. The different types of GC include None, Partial, Sticky, Full, and Max.<sup>1</sup> First, none is a GC type when no GC is triggered. Then, Sticky is the type of GC that attempts to only free objects allocated since the last GC. The Partial GC is the GC type that marks the application heap but not the Zygote. The Full GC is the GC that marks and frees in both the application and Zygote heap. And finally, the Max GC identifies the different GC types that occurred.<sup>1</sup> This research primarily focuses on the thread running the GC iteration. The GC thread running on further examination identifies various parameters like the type of objects collected and the cause for GC from the dump acquired. This dump helps us determine what objects get collected in each GC type when analyzed with userland forensic tools. Finally, the research will develop an evaluation platform to enable forensic investigators to present the type of objects deleted from memory. The retrieved objects from userland forensic tools and the GC type identified with this approach can convince the jury that the objects deleted may or may not be critical in forensic investigation.

In newer Android® versions, ART allocates objects using RegionSpace and LargeObjectSpace allocators that trigger the GC for efficient memory utilization.<sup>2,3</sup> The RegionSpace allocator allocates small objects like primitive objects, and the LargeObjectSpace allocator allocates large objects like multimedia in memory.<sup>2,3</sup> If the application creates many objects, then the Android® Runtime environment triggers GC frequently.<sup>2</sup> As a result, the type and number of objects allocated and deleted during the runtime, the cause, and the type for GC to occur are unknown to forensics investigators. Thus, the impact of GC type triggered by the runtime is the primary focus of this research.

This presentation evaluates acquired memory dumps with tools like DroidScraper and AmpleDroid in recovering objects from RegionSpace and LargeObjectSpace, respectively.<sup>2,3</sup> In this work, the memory dumps acquired from the same application are analyzed in-depth with the tools mentioned above to monitor the number of objects, the GC type, and the cause for GC to get triggered. The results demonstrated that different objects were collected and lost in different GC types; for example, dumps acquired when Full GC type occurred retrieved fewer objects than Sticky or Partial GC type. Hence, this study's hypothesis opens a new research dimension to explain the correlation between the GC and object recovery to identify which objects get removed during each GC type. The research illustrates how forensic investigators can convince the jury that the type of GC that occurred has or has not significantly impacted forensic analysis on a multi-app platform such as Android®.

#### Reference(s):

1. [http://androidxref.com/8.0.0\\_r4/xref/art/runtime/gc/collector/gc\\_type.h#28](http://androidxref.com/8.0.0_r4/xref/art/runtime/gc/collector/gc_type.h#28).
2. Ali-Gombe, A.; Sudhakaran, S.; Case, A.; Richard, G.G. III (2019). DroidScraper: A Tool for Android In-Memory Object Recovery and Reconstruction. In *22nd International Symposium on Research in Attacks, Intrusions, and Defenses ({RAID} 2019)* (pp. 547-559).
3. Sudhakaran, S.; Ali-Gombe, A.; Orgah, A.; Case, A.; Richard, G.G. III (2020, December). AmpleDroid recovering large object files from Android application memory. In *2020 IEEE International Workshop on Information Forensics and Security (WIFS)* (pp. 1-6). IEEE.

#### Garbage Collection, Object, Recovery



## C25 The Forensic Use of Sparse Files

*Mark Guido, MS\*, MITRE, McLean, VA; Justin Grover, MS, MITRE, McLean, VA*

**Learning Overview:** The goal of this presentation is to introduce and describe a little-known method of representing a large file that has the potential to provide great benefits to the digital forensics community, allowing many existing forensic tools to operate normally on that large file.

**Impact Statement:** This presentation will impact the forensics community by providing the method and associated utilities that are now default in most modern operating systems that can help an organization save significant storage resources, leading to both time and money savings.

Linux® systems running kernel version 3.1 (released in October 2011) or later maintain near-universal support for sparse files. Sparse files are special files that have large amounts of space pre-allocated to them but do not use the full amount of space from a host's file system. Modern Linux® utilities handle these files; however, the use of sparse files in the field of digital forensics remains an underused and poorly understood concept. Common tools in a forensic examiner's toolkit, such as md5sum and dd, operate on sparse files like they would on a regular file with some counterintuitive results. Using md5sum, a regular file's MD5 hash matches that of its sparse counterpart. Similarly, the dd tool outputs the representative regular file when provided a sparse file as its input.

In a forensics laboratory with requirements for long-term storage, sparse files, if used correctly, may provide an organization with a potential for significant storage costs savings. So, if this is the case, why are sparse files not used more frequently in forensics? This presentation will attempt to answer questions concerning the use of sparse files: What are the advantages and disadvantages for using sparse files in forensics? Are there similarities and differences in the use of sparse files on Linux® EXT4, Windows® NTFS, and macOS® X APFS? Is it possible to transmit data in a sparse file format, limiting the amount of data sent from the sender to the receiver? Do mainstream forensics tools operate in the same manner on sparse files and their regular counterparts?

As an example of potential cost savings, this study conducted an experiment in 2013 at Purdue University, where over 1,100 forensic images of Samsung™ Galaxy S3 smart phones were collected. 1 Human test subjects used the smart phones normally for almost a semester and the smart phones attempted to deliver iterative forensic images each day. At the end of the experiment, the images were reconstituted, totaling more than 17TB of data collected. The analysis showed that over 40% of the data acquired were blocks of zero bits in unallocated space on these images. How much more storage costs and time savings could have been saved by properly utilizing sparse files to reconstitute the images?

### Reference(s):

1. Guido, M.; Brooks, M.; Grover, J.; Katz, E.; Ondricek, J.; Rogers, M.; Sharpe, L. (2016). Generating a Corpus of Mobile Forensics Images for Masquerading User Experimentation. *Journal of Forensic Science*, 61(6), 1467-1472.

### Sparse, Preallocated, Representation

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### C26 Google® Chromebook™ and Takeout™ Forensics: A Comparative Analysis of Data Recovered from a Logical Acquisition of a Chromebook™ as Compared to Data from a Google® Takeout

*Jessica Hyde, MS\*, Magnet Forensics, Herndon, VA*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of acquiring data from Chromebook™ and understand how the data available differs from the data available from a Google® Takeout™.

**Impact Statement:** This presentation will impact the forensic science community by showing the types of data available on Google® Chromebook™.

Chromebook™ sales have risen dramatically year-over-year with a 68.6% increase for the 2nd quarter of 2021.<sup>1</sup> The need to investigate Chromebook™ has recently been aided by the Chromebook™ Forensic Acquisition method released by Daniel Dickerman, making acquisitions of Chromebook™ accessible.<sup>2</sup>

Previous analysis looked at some of the data available on the Chrome OS™ by looking at data left behind on Chromium™ OS.<sup>3</sup> This presentation will explore the data available from a Google® Takeout™ and a Google® Chromebook™ logical acquisition using the Daniel Dickerman method for the same account and compare the available data detailing the data locations for both sources.<sup>2</sup>

The goal of this research is to demonstrate that there are artifacts available from the Google® Chromebook™ that are not available from cloud-based acquisitions, such as a Google® Takeout™. It is hoped that this research will encourage digital forensic examiners to acquire data from Chromebook™ when conducting forensic examinations.

**Reference(s):**

1. International Data Corporation (IDC). (2021, July 30). Chromebook and Tablet growth continued in the second quarter Despite on-going Supply Concerns, according to IDC. IDC. <https://www.idc.com/getdoc.jsp?containerId=prUS48120621>.
2. Dickerman, D. (2020). Chromebook Forensic Acquisition. DFIR Review. Retrieved from <https://dfir.pubpub.org/pub/inkjsqrh>.
3. Hyde, J.L.; Saliba, J. (2018, October). Chrome Nuts and Bolts: Chrome OS / Chromebook Forensics. OSDfCon. Herndon, Virginia.

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**Chromebook™, Takeout™, Chrome OS™**

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## C27 The Examination of Questioned Digital Documents Customized From a Database

*Oluwasola Mary Adedayo, PhD\**, The University of Winnipeg, Winnipeg, Manitoba, Canada; *Martin S. Olivier, PhD*, University of Pretoria, Pretoria, Gauteng, South Africa

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**Learning Overview:** The goal of this presentation is to describe novel approaches to examine digital documents that are customized and derived from a database.

**Impact Statement:** This presentation will impact the forensic science community by drawing attention to the examination of digital documents and describe approaches for examining customized digital documents derived from database systems.

The trend toward digitalization means that digital documents with intrinsic value are continually created. Whereas questioned document examination often relies on the examination of physical artifacts, approaches for examining questioned digital documents need to be improved.

Questioned digital documents may originate from different sources. A possible scenario is where a customized or personalized document derived from a database is questioned. Often an original document is created via a template that is populated with information in the database and is deemed to be the authoritative version of the document. The examiner is faced with the challenge of determining whether the document was derived from the template and the database instance at the time the document was created. Examination is based on comparing the document to the template, dependence of document fields on other database fields, and reconstruction of the database to the creation time of the document (if necessary).

The examination process may depend on the size and quantity of documents to be examined. Examination of isolated documents with minimal content may be performed manually by attempting to determine the values extracted from the database at the time of interest. Examination of multiple documents and/or documents with large amounts of data (e.g., account statements listing many transactions) will benefit from techniques to recreate the documents for comparison. This presentation discusses some approaches and challenges that apply to such a context.

The complexity of recreating the original document(s) from the database depends on the availability of the template from which the document was derived and the ability to determine the content of the database at the time the document was derived. Three possibilities were considered: (1) when the template previously used for the document creation exists, the original document(s) can be reconstructed if the database can be reverted to the same instance as when the document was created and the comparison process that can be automated; (2) when the document template has been modified, but still communicates the same information as before, the reconstructed document may differ from the questioned document, but the data extracted from the database should remain the same. Comparing the two documents may involve suppression of “constant” text and exploring differences in the remaining data, and (3) when the template is lost, the examiner may use the database schema and the questioned document to determine inferred values that may then be compared with those on the questioned document. It is important to note that when the database schema has changed or the database needs to be reverted, some information may be overlooked or not be available for comparison.

This presentation assumes that the database or schema can be reverted to the time that the questioned document was (purportedly) created. Although this research was not focused on reverting or reconstructing the database, approaches that have been described for this purpose can be used to obtain an earlier instance of the database for document examinations.<sup>1,2</sup>

### Reference(s):

1. O.M. Fasan; M.S. Olivier. Reconstruction in Database Forensics. *Advances in Digital Forensics VIII*. Springer, 2012, pp. 273–287.
2. O.M. Adedayo; M.S. Olivier. Schema Reconstruction in Database Forensics. *Advances in Digital Forensics X*. Springer, 2014, pp. 101–116.

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### Questioned Digital Documents, Database Forensics, Reconstruction

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### C28 A Comparison of Apple® iPhone® Extraction Methods for Multimedia

*Jeff Smith, MS\*, MITRE, McLean, VA*

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**Learning Overview:** After attending this presentation, attendees will be informed regarding basic and advanced techniques for the analysis and comparison of images, video, and audio and the effects of various transfer methods on multimedia originating from Apple® devices with iOS® 13 and iOS® 14.

**Impact Statement:** This presentation will impact the forensic community by laying out the challenges in the extraction and comparison of multimedia files; demonstrating the effects of various seemingly innocuous transmission and sharing methods on multimedia; and justifying the need for continued study and documentation on the effects of transmission, sharing, and extraction methods on multimedia files.

In multimedia forensics, it is necessary to obtain test samples from known devices in order to conduct hypothesis testing on evidence. This can be necessary in various tasks, including authentication of media where one substantiates “that the data is an accurate representation of what it purports to be.”<sup>1</sup> This becomes important when testing a recording’s originality or evaluating its relationship with the device purported to have created it. It is up to the forensic scientist to obtain known samples from the specific device or devices for testing. Samples can be collected in various ways: from public datasets or other internet sources that have samples from the same make/model device, by obtaining the device and collecting the necessary material, or by request from the contributor of evidence who may be the victim, suspect, or other witness. In any case, collecting samples in their original form or the same manner that the evidence itself was obtained from the device is crucial. This task becomes complicated when analyzing material from mobile devices that can transmit and transfer multimedia files in a number of ways such as Bluetooth® connectivity, wired transfer to PC, Multimedia Messaging Service (MMS) native to the device, or by apps such as WhatsApp, email, file sharing apps like Dropbox, etc.

If the goal is to obtain test samples in their most original form, then collecting data from a forensic image or backup of the device is the best method. However, this is not always possible, practical, or necessary. Furthermore, when working with evidence in blind without knowledge of the originating device, an examiner relies on metadata such as EXIF data fields showing make/model or other unique entries. But in practice, while one field is consistent with what one would expect to see from a particular device, other fields may not be. While inconsistencies could arise from image or audio manipulation and attempted obfuscation, they could merely be the result of routine file sharing or transfer. Which transfer methods provide a bit-for-bit duplicate to what was created and stored on the device? Which methods modify or recompress data and how? As a baseline to further study and public documentation, the research presented here seeks to provide insight into these questions regarding Apple® iOS® 13-15 devices.

**Reference(s):**

<sup>1</sup> Digital & Multimedia Evidence Glossary, v3. *Scientific Working Group on Digital Evidence (SWGDE)* 2016. [www.swgde.org](http://www.swgde.org).

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#### Multimedia, Digital, Comparison

### C29 CoreDuet, KnowledgeC, and InteractionC: Just How Much Does My iPhone® Really Need to Know About Me?

*Joseph Levi White, PhD\*, Defense Forensic Science Center, Forest Park, GA*

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**Learning Overview:** After attending this presentation, attendees will understand the types of real-world user activities mobile devices track/log and how these activities may be used to generate a timeline for investigations. This presentation will present information through a case study from an investigation completed at the Defense Forensic Science Center's (DFSC) United States Army Criminal Investigation Laboratory (USACIL).

**Impact Statement:** This presentation will impact the forensic science community by providing insight into the application of data extracted from mobile devices, specifically the KnowledgeC and InteractionC databases on Apple® iPhones®, on determining a user's digital device interactions and real-world activities. This presentation will provide an example of how this information was used to provide critical information in a recent USACIL case.

One of the primary roles of a digital forensic examiner is using the data extracted/copied from submitted digital and multimedia evidence to outline a user's activities regarding an alleged incident under investigation. For analysis of mobile devices (cell phones, tablets, etc.), these activities have traditionally included text-based communication (text messages, email, third party messaging applications), call log entries, Global Positioning System (GPS) information, camera usage, and other types of data one would traditionally associate with the use of a mobile device. However, the past several years have seen great advancements in technology and hardware, along with a large expansion of the types of data that are logged on mobile devices. A few specific log files have proven to be a repository of a great amount of data useful to forensic analysts and investigators, including the KnowledgeC and InteractionC databases retained on Apple® mobile devices.

These databases are not exclusive to Apple® mobile devices but are also found on other Apple® devices, such as laptops and computers. No official explanation of the CoreDuet management system could be located. However, the understood intent of this tracking system is to allow for monitoring and management of internal components of mobile devices and computers and to allow for handoff between multiple Apple® devices utilized by the same user. The CoreDuet system maintains several databases containing data of potential forensic significance, including the KnowledgeC and InteractionC databases.

There is a multitude of data types that may be retained within the KnowledgeC and InteractionC databases on Apple® devices. This presentation will utilize a recent digital and multimedia evidence analysis completed at the USACIL as an outline for presenting data that may be retained within KnowledgeC and InteractionC database files. This case study provides an example of how something as simple as the battery percentage displayed on the device and whether or not the device was connected to a charger can provide enough detail to make or break a case of alleged sexual assault. This case will highlight the importance of examining system-managed database files when determining an exact timeline of events surrounding an alleged crime, or if it was even possible for the alleged crime to have happened at all.

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#### Activity Tracking, Mobile Forensics, User Activity

## C30 SQLite Data Recovery

*Richard Ayers, MS\*, National Institute of Standards and Technology, Gaithersburg, MD; Jenise Reyes, BS, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** After attending the presentation, attendees will better understand the SQLite Data Recovery Specification, Test Assertions, and Test Cases document. The specification provides the forensic community with a documented set of test cases for basic methods of recovering and reporting evidence as contained within SQLite databases. The document defines SQLite forensic data recovery tool requirements. These requirements are used to derive test assertions, statements of conditions that are then checked after a test case is run. Each test assertion is covered by one or more test cases consisting of a test protocol and the expected test results. The test case protocol specifies detailed procedures for setting up the test, executing the test, and measuring the test results.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of the SQLite Data Recovery specification and associated requirements, test assertions, and test cases.

There is a critical need in the law enforcement community to ensure the reliability of computer forensic tools. A capability is required to ensure that forensic tools consistently produce accurate, repeatable, and objective test results. The goal of the Computer Forensic Tool Testing (CFTT) project at the National Institute of Standards and Technology (NIST) is to establish a methodology for testing computer forensic tools by the development of functional specifications, test procedures, test criteria, and test sets. The results provide the information necessary for toolmakers to improve tools, for users to make informed choices about acquiring and using computer forensics tools, and for interested parties to understand the tools' capabilities. This approach for testing computer forensic tools is based on well-recognized international methodologies for conformance testing and quality testing.

The scope of this specification is limited to software tools capable of presenting recovered data stored within SQLite databases. This also includes stand-alone SQLite forensic tools that provide additional functionality. This specification is general and capable of being adapted to other database formats should they become more widely used. The intended audience for this document is forensic examiners with an existing basic knowledge of SQLite. This document does not address every detail about how SQLite works. Rather, the scope of this material is at a higher level.

The goal of SQLite Data Recovery Specification is to provide the forensic community with Requirements, Test Assertions and Test Cases for testing a digital forensic tool tailored for reporting and recovering SQLite data artifacts.

The presentation gives an overview of the SQLite Data Recovery Specification, Test Assertions, and Test Cases document.

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### Digital Forensics, SQLite, Data Recovery

## C31 A Forensic Analysis of the Viber Instant Messaging Application on iPhone® Devices

*Akshata Thole, BTech\**, Purdue University, West Lafayette, IN; *Nina Matulis, BS*, Purdue University, West Lafayette, IN; *Marcus Rogers, PhD*, Purdue University, West Lafayette, IN

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the forensic artifacts of the Viber instant messaging application on iPhones® that are recovered from the iTunes® backup using various forensic tools and manual analysis. Attendees will also have a better understanding of the time frame of application installation to uninstallation on iPhone® devices.

**Impact Statement:** This presentation will impact the forensic science community by providing law enforcement officials with a guide to determine a time frame for the usage of the Viber application on iOS® devices. Additionally, law enforcement officials will gain a better understanding of the reliability of the forensic tools.

Among the various free messaging applications, the Viber application is one of the most widely used worldwide. Viber can be downloaded for free on Android®, iPhone®, iPad®, Windows® PC, Mac®, and Linux® devices, making it easily accessible. Analysis of the Viber application from a forensic standpoint is crucial as the app could be used as a method of communication for illegal actions as it can be easily uninstalled, therefore hiding any evidence of communication. Additionally, there are no current publications on recovering forensic artifacts from the Viber application on iOS® devices. There are also no publications related to determining the date and time when any application was deleted on iOS® devices. The inability to determine the exact date and time that an application was deleted from an iOS® device was the motivation for this research. If the date and time that the Viber application was deleted from iOS® can be determined, it is likely that the same information could be located for any application on an iOS® device.

The focus of this presentation is to provide the results of the analysis of the artifacts left behind by the Viber application in the iTunes® backup for iPhones® running iOS® 14 and determine if the date and time of uninstallation can be determined. The Viber application has three different installation states:

1. Installed and in use – The Viber app in frequent use by the user
2. Installed but removed from Home Screen – The Viber app is still in the user's App Library, however, not visible on the Home Screen
3. Uninstalled

The iTunes® backup from the different installation states will be analyzed using the following tools: (1) Magnet AXIOM®, (2) Cellebrite BlackLight®, and (3) reincubate iPhone® Backup Extractor®. Manual analysis will also be completed using (1) iBackup Viewer® and (2) DB Browser for SQLite®.

This study will analyze the forensic artifacts from the iTunes® backup for the Viber application that each of the forensic tools found and determine if the date and time that the app was fully uninstalled can be determined. Additionally, there will be a comparative analysis of each of the forensic tools to determine if there are any discrepancies between what artifacts each tool has found.<sup>1</sup> A comparative analysis between the artifacts that the forensic tools recovered versus the manual analysis will also be completed to determine the tools' reliability.

### Reference(s):

- <sup>1</sup> Hughes, N.; Varol, C. (2020, March). The Critical Need for Tool Validation before Using Malware Scanners in Digital Forensics. In ICCWS 2020 15th International Conference on Cyber Warfare and Security (Vol. 228). Academic Conferences and publishing limited.
2. Viber Forensics, iOS® Application Uninstallation, Forensic Tool Reliability

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### Forensic Tool Reliability, iOS® Application Uninstallation, Viber Forensics

### C32 Forensic Analysis on Cryptocurrency Wallet Apps

*Chen, Shi, MS\*, Iowa State University, Ames, IA; Yong Guan, PhD, Iowa State University, Ames, IA*

**Learning Overview:** After attending this presentation, attendees will have learned the security issues discovered from cryptocurrency wallet apps as well as the type of digital evidence generated and associated location where the evidence is stored.

**Impact Statement:** This presentation will have an immediate impact on the forensic science community, in particular, digital forensic practitioners and prosecutors who have a casework investigation involving the use of cryptocurrency wallet apps or any other parties that have a general interest in the cryptocurrency wallet apps .

This project aims at conducting forensic analysis on a large scale of cryptocurrency wallet apps to discover all the digital evidence (i.e., Universally Unique Identifier [UUID], location) stored in the local file system or sent over to a remote server. Due to the influence of the COVID-19, cryptocurrency wallet apps are gaining increasing demand and accelerating the shift from cash to digital options. According to the statista, the ten most popular cryptocurrency wallets have been downloaded 2.2 million times in December 2020 and have grown to over 5.6 million one month later.<sup>1</sup>

As with many investigations, location and timestamp information is vital to solving crimes, which could serve as crucial evidence to determine whether a suspect was at the scene when the crime was committed. Visited URLs, browser cookies, and bookmarks offer a wealth of information on user activities, where forensic practitioners could utilize them for investigations involving cryptocurrency transactions. In addition, usernames, passwords, and wallet addresses may be found in the browsing history as well. Additionally, user text inputs provide valuable information for forensic investigations. There may be keyword searches or searches for specific wallet addresses or crypto transactions (e.g., visits to blockchain.com [Bitcoin]). Therefore, we collect thousands of related source Application Programming Interfaces (APIs) targeting four groups of evidence types when conducting the analysis of cryptocurrency wallet apps .

This study's technique is based on the effective toolkits developed and published at the 12th Institute of Electrical and Electronics Engineers (IEEE) Systematic Approaches for Digital Forensic Engineering (SADFE 2018) and the Association for Computing Machinery (ACM) Conference on Computer and Communications Security (CCS 2018). This approach examines both system logs and file systems. This study collected 170 Android® cryptocurrency wallet apps from 57 different app markets worldwide. The analysis result demonstrates that, through this proposed approach, 157 of the 170 cryptocurrency wallet apps have been discovered to create various types of evidence and to store the information in local storage. This study identified 272 evidence records with evidence type and associated file path, which assisted forensic examiners with the digital investigation of Android® cryptocurrency wallet apps. This work demonstrates that 128 apps collect and store user private information, including location, timestamp, visited URL, etc.

According to the analysis result, 15 out of 170 cryptocurrency wallet apps (e.g., Bitcoin Wallet, Blockchain.com Wallet, Dogecoin Wallet) utilize BitcoinJ, a Bitcoin client library that is used to communicate with Bitcoin networks. However, this analysis experiment confirmed that Bitcoin wallet applications, which contain the BitcoinJ client library, store the user Bitcoin addresses in the app's internal storage. In addition, this study could retrieve Bitcoin transaction history from the system log for the apps mentioned above.

#### Reference(s):

- <sup>1</sup> Best, R. de. (2021, July 29). Cryptocurrency wallet downloads 2021. *Statista*. <https://www.statista.com/statistics/1206516/global-cryptocurrency-app-downloads/>.

#### Mobile Forensics, Cryptocurrency Wallet, Digital Evidence



### C33 Memory Forensic Analysis for Programmable Logic Controllers (PLCs)

*Muhammad Rais, MS\*, Virginia Commonwealth University, Richmond, VA; Irfan Ahmed, PhD, Virginia Commonwealth University, Richmond, VA*

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**Learning Overview:** After attending this presentation, attendees will understand the important forensic artifacts available in a PLC's memory dump and a process to extract them rapidly without involving tedious reverse engineering of proprietary firmware.

**Impact Statement:** This presentation will impact the forensic community by discussing a memory forensic methodology for PLCs referred to as DeepPLC. Instead of relying on firmware reverse engineering, DeepPLC generates the memory profile through planned test cases involving common application layer interaction with a test PLC. It then utilizes the profile to analyze the memory dump to extract forensic artifacts.

The PLCs, the nucleus of most Industrial Control Systems (ICS), directly monitor and control physical processes such as nuclear plants and power grid stations through a closed-loop control system involving sensors and actuators. Due to the central role of PLCs in ICS, they are often subject to a cyberattack to sabotage critical infrastructure. The memory forensic analysis of a suspect PLC can help answer questions about a cyberattack, such as the presence of malicious firmware, control logic injection, and manipulation of Input/Output (I/O) devices (e.g., sensor and actuators). In the current state, the PLC market is full of heterogeneous hardware architecture, proprietary firmware, and control software, making their memory forensics a challenging task. To address this research problem, this presentation proposes a memory forensic methodology for PLCs referred to as DeepPLC. Instead of relying on firmware reverse engineering, DeepPLC generates the memory profile through planned test cases involving common application layer interaction with a test PLC. It then utilizes the profile to analyze the memory dump to extract forensic artifacts. The profile is a formalized set of rules generated by discovering data structure definitions and connecting graphs identified throughout the process.

The aim of presenting DeepPLC methodology is to facilitate the community of interest (such as ICS owners, operators, forensic investigators, and vendors) to generate memory analysis tools for different PLCs. This presentation further discusses a case study of DeepPLC on a real-world PLC, Allen-Bradley 1756-L61 controller. The resultant profile successfully discovers the control logic (the control application running on the PLC), the state of sensors and actuators available in the physical process, PLC configuration data, and other forensically beneficial logs from the memory dump. The output of the case study, presented as a Python® library and shared with the community, can be readily used for the memory forensic analysis of the same model's controller.

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**PLC, Memory Forensics, CPS**

### C34 The Analysis of a Digital Diary to Aid in a Child Sexual Assault Investigation: A Case Study

*Joseph Levi White, PhD\*, Defense Forensic Science Center, Forest Park, GA*

**Learning Overview:** After attending this presentation, attendees will understand the background functionality of Microsoft® (MS) Word® document recovery features and how these features allowed examiners at the Defense Forensic Science Center's (DFSC) United States Army Criminal Investigation Laboratory (USACIL) to provide investigators solid evidence in a case of alleged sexual assault of a minor.

**Impact Statement:** This presentation will impact the forensic science community by providing knowledge into the document recovery features of MS® Word®. This presentation will outline how analysis of a digital sexual diary maintained by the perpetrator was used in a child sexual assault investigation and provide insight into the sexual background and mindset of the document's author.

In 2012, several items of digital and multimedia evidence were submitted to the USACIL for analysis in an alleged sexual assault of a minor case. Items of evidence included a laptop computer, an external hard drive, and a mobile phone. The examiner was requested to recover all information related to the alleged sexual assault of a specific underage individual, but data located on the submitted external hard drive revealed much more than investigators originally understood about the case.

In addition to pictures and videos, contact information, messaging content, website profile information, and much more associated with the underage female, a very organized presentation of data regarding additional females were observed. This included an organized file structure, with folders named after individual females, each of which contained files such as documents regarding the individual, and pictures and/or videos detailing the sexual activities with the named female.

According to the submitter's information (interviews, etc.), the accused indicated a belief that the female who was the focus of this investigation was age 19 at the time of the incident. Copies of web pages from the submitted computer indeed showed how the underage female had apparently misrepresented her age as 19 online. The accused admitted to having sexual intercourse with the female, as proven through photographic and video evidence he maintained on the external hard drive, but did not admit to knowing her to be underage at the time.

One of the most important pieces of data recovered in this case was an MS® Word® document detailing the apparent sexual activities of the author, from childhood (13 years old) to 2011. This document detailed sexual encounters with over 350 females. Entries into the document were in chronological order and contained a name, age of the female, number of sexual encounters with that female, codes indicating specific sexual activities, ethnicity, height, weight, physical description, and a summary sentence regarding the female. It was noted that several females on the list were described as being under the legal age, but these were primarily listed as occurring in a timeframe of when the accused was also underage. However, at the time of the incidents under investigation, the accused was currently over 30 years of age and the victim was age 14.

MS® Word® maintains a recovery feature that allows users to not lose data in the case of a system/power failure. Two recovery copies of the aforementioned sexual diary were located by the examiner. A comparison of these documents showed a methodical change of information related to several females on the list, changing them from being underage to over the age of 18. Also modified between versions of the document was the actual name and age of the victim in this case. This information, coupled with the video evidence and additional case information, showed active knowledge of the victim's age at the time of the sexual acts.

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#### Sexual Deviancy, Document Modification, Data Recovery

## C35 Sextortion: Understanding Perpetrator Behavior and the Digital Evidence Available to Investigators

*Martin Novak, MPA\*, National Institute of Justice, Washington, DC; Danielle Crimmins, MS, University of Baltimore, Baltimore, MD; Kathryn Seigfried-Spellar, PhD, Purdue University, West Lafayette, IN*

**Learning Overview:** After attending this presentation, attendees will understand: (1) types of sextortion; (2) the characteristics and psychology of individuals who engage in sextortion; and (3) the potential digital evidence involved in sextortion cases.

**Impact Statement:** This presentation will impact the forensic science community by: (1) addressing the types of digital evidence that can assist a sextortion case; and (2) providing an overview of the characteristics and psychology of individuals who engage in sextortion.

One devastating impact of COVID-19 reported around the world was the increase in child sexual exploitation offenses—including sextortion.<sup>1-3</sup> More than 86 million reports of suspected child sexual exploitation have been made to the CyberTipline between 1998 and 2020.<sup>4</sup> However, of that 86 million, 21.7 million reports were made in 2020 alone—a 28% increase from 2019.<sup>4</sup> The majority of these reports were for “apparent child sexual abuse material, online enticement (including sextortion), child sex trafficking, and child sexual molestation” (para 2).<sup>4</sup> In addition, from 2017 to 2018, the Federal Bureau of Investigation (FBI) reported a 242% increase in extortion-related complaints, with the majority being sextortion.<sup>5</sup>

Sextortion is a form of “cyber extortion in which an offender demands that victims provide them with sexual images, sexual favors, or other things of value and threatens to harm or embarrass victims if they fail to comply.”<sup>6</sup> Previous research has identified four types of sextortion offenders, which include: (1) Minor-focused; (2) Cyber; (3) Intimately Violent; and (4) Transnational.<sup>7</sup>

The goal of the current study is to examine three research questions: (1) What types of digital evidence is involved in sextortion cases?; (2) What are the characteristics and offender psychology of individuals engaging in sextortion?; and (3) Do any of the four types have commonalities between them? To answer these research questions, the current study examined the literature, relevant laws and court cases, and criminological and psychological theories.

In an effort to assist prosecutors and investigators, challenges associated with investigating and prosecuting sextortion cases are also addressed.

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### Digital Evidence, Sextortion, Offender Psychology

## D1 The Characterization of Disperse Dyes in Polyester Fibers Via Direct Analysis in Real Time (DART®) Ionization Mass Spectrometry

Zoe Millbern, BS\*, North Carolina State University, Raleigh, NC; Nelson Vinueza, PhD, North Carolina State University, Raleigh, NC

**Learning Overview:** Attendees can expect to learn a simple, straightforward method for analyzing polyester fibers via mass spectrometry without degradation or excessive sample preparation of potential evidence. Spectra and ion distributions for disperse dyes (one of the most widely applied colorant classes) will be explained and displayed, and the reasoning behind methodology decisions will be covered.

The methodology covered allows for rapid, sensitive analysis of evidence without damaging fibers, allowing for potential application to mobile instrumentation. A collection of spectra for 30 dyes presents a start for a potential database allowing for immediate/rapid identification of colorants.

**Impact Statement:** This presentation will inform attendees of an application for DART® ionization in trace fiber analysis by explaining how this methodology can be used to identify disperse dyes accurately and quickly in polyesters *in situ* via high-resolution mass spectrometry. A collection of disperse dye spectra and structures has been initiated as part of a proposed database allowing for rapid identification and comparisons between evidence and known contributors. The methodology presented highlights not only for DART® ionization as a non-destructive analysis for textiles, but also for its ability to analyze multiple colorants simultaneously.<sup>1</sup>

Polyester and other synthetic fibers are found in clothing, carpets, upholstery, and several other everyday sources, so the chance of finding these fibers at the scene of crime are relatively high.<sup>2</sup> A good portion of these fibers likely contain some sort of colorant and, in the case of polyester and similar fibers, the most common colorants used are disperse dyes. Disperse dyes are generally inert molecules with a chromophore and structural resonance that promotes the excitation of light of a particular wavelength, and they can be separated into low-, medium-, and high-energy classes depending on their size. In trace evidence analysis, fiber samples may be small and need to be retained for additional testing, and a non-destructive methodology or one that minimally affects the evidence is advantageous.

DART® ionization involves a stream of heated, metastable gas that can provide instantaneous, real-time results, and with a relatively simple construction may translate well to a portable, hand-held device.<sup>3</sup> The ionization mechanism employed by this methodology will be covered, specifically describing the process involved when ionizing the dyes, and informing attendees how the method works overall in a way that is applicable to other materials. Another significant advantage to DART® is the lack of sample preparation required, so an applicable fiber can be held in the ionization stream directly without further manipulation. Due to the fact these dyes (and in the case of Polyester Terephthalate [PET], the fibers) are inert, the fibers must be thermally swelled to open the structure and physically entrap the dye molecules. Therefore, analysis via a heated stream of gas, as with DART® ionization, works well for physically dislodging dye molecules. Acid and fiber reactive dyes are chemically bound to fiber, making them significantly more difficult to dislodge, and the optimum temperature for this methodology will consider the melting points of other fibers as well.

PET fabrics dyed with a known red, yellow, and blue dye with different chromophores and structural characteristics were generated, along with a trichromatic mixture generating a black fabric. Fiber and fabric samples of varying sizes were taken from each fabric and presented to the DART® gas stream in different manners (fiber and fabric via tweezers, mounting on an IonSense® mesh card, and extraction solutions) and at different temperatures and distances to generate a commonly applicable methodology. Once the mass spectrum for each color was confirmed, the black mixture was evaluated, and it was determined that each dye present was detectable. While these four samples will be presented as examples, the methodology was applicable and used to generate comparable high-resolution spectra for 30 disperse dyes.

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### DART®, Dyes, Fibers

## D2 Portable Dynamic Vapor Microextraction (P-DVME) for Forensic and Environmental Field Studies

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**Learning Overview:** After attending this presentation, attendees will better understand the concept design and development of a P-DVME sampling device for forensic studies and environmental monitoring. Attendees will also learn about the design process, including heat transfer considerations, power budgeting, and other crucial aspects required for a robust and functional device comparable to those used in a laboratory environment.

**Impact Statement:** This presentation will impact the forensic and environmental science community by exposing attendees to a novel field sampling approach using P-DVME. P-DVME has many advantages compared with other approaches (e.g., Solid-Phase Microextraction [SPME] fibers) owing to the ease of sample collection, sensitivity, and the ability to collect vapor without *a priori* knowledge of the species present.

Field-deployable diagnostics for trace vapor analysis have seen widespread adoption in the forensics and environmental monitoring communities due to numerous technological advancements over the past several years. Reduction of the cost and size of field-deployable analytical instruments is advantageous to field sampling and analyses, yet it is still not possible to match the sensitivity, repeatability, and detection limits of lab-based measurement approaches. Therefore, field-deployable sample collection for later analysis is appealing owing to the ability to utilize existing lab-based analytical instruments and techniques in tandem with relatively simple portable collection devices. One technique for vapor sample collection that uses adsorbent-coated capillary columns for sample preconcentration is Porous Layer Open Tubular-Cryoadsorption (PLOT-Cryo). This technique has been applied extensively in the lab to the analysis of fire debris, explosives detection, grave soil analysis, and vapor pressure measurements.<sup>1-4</sup> The PLOT-Cryo approach works by collecting vapors on an adsorbent layer within a chilled (~ 0°C) capillary column. This technique is fast, sensitive, and does not require large amounts of sample.

Building on the success of the lab-based approach, a portable device was developed and deployed in the field.<sup>5,6</sup> The device has many advantages compared to alternative approaches, such as the ability to elute the sample in the field and, by utilizing many capillaries with different adsorbents, the ability to capture vapor samples without *a priori* knowledge of the analytes of interest. The device utilizes vortex tubes for temperature control of the capillary and a vacuum generator to sweep vapor samples through the capillary for collection. The performance was comparable to that of lab-based devices, but the use of vortex tubes and a vacuum generator required the use of an air compressor, which limited portability.

The device was redesigned to achieve true portability and is based on a much-improved laboratory dynamic headspace sampling apparatus—Dynamic Vapor Microextraction (DVME)—recently used to measure the vapor pressure of eicosane with orders of magnitude lower uncertainty compared with the PLOT-Cryo technique. The next generation portable headspace sampling device concept was designed in parallel with DVME. This new device abandoned the use of compressed air for cooling and vacuum generation in favor of a battery-powered design that utilizes a high-efficiency thermoelectric cooler for capillary vapor trap cooling and a positive displacement pump to generate flow. Additionally, an improved user interface that simplifies sample collection and tracking, and advanced data logging features for the capture of environmental parameters to further enhance the device's capabilities will be incorporated. A detailed explanation of the concept design, including heat transfer considerations and component selection, as well as future field studies are presented.

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### Field Deployable, Vapor Sampling, Microextraction

## D3 The Use of Atmospheric Pressure Ionization (APPI) Coupled with Mass Spectrometry (MS) to Increase Sensitivity for the Identification of Disperse Dyes in Trace Evidence

Morgan Demmler, MS\*, North Carolina State University, Raleigh, NC; Nelson Vinueza, PhD, North Carolina State University, Raleigh, NC

**Learning Overview:** After attending this presentation, attendees will learn about the differences between APPI and Electrospray Ionization (ESI) MS and how each ionization technique affects instrument sensitivity of disperse dyes, enhancing forensic analysis of trace fibers.

**Impact Statement:** This presentation will impact the forensics community by providing a novel way to thoroughly examine trace fibers of extremely small sizes by high-resolution mass spectrometry.

Textiles play a major role in connecting places, objects, and people to a crime scene. Oftentimes, small fibers can be found at a crime scene when a person's body or clothing comes into contact with a textile. This follows Locard's Principle, which states that when two objects come into contact with each other, there will be an exchange of microscopic material.<sup>1</sup> There are many different kinds of textiles that can be found at a crime scene. These include clothing, carpets, upholstery, and others. Trace evidence of textiles comes in the form of microscopic pieces of fiber. As with all trace evidence, a challenge arises due to the extremely small nature of this evidence. While microscopy and spectrophotometry do not require a large sample size, these techniques can only give very limited information on dye types.<sup>2</sup> Therefore, it is important to find more sensitive analytical techniques that can be successfully performed on microscopic samples.

Second to cotton, polyester is the most commonly produced textile. In addition, it accounts for 75% all synthetic fibers manufactured globally.<sup>3</sup> Disperse dyes are commonly used to dye polyester textiles. In this study, three commercially available disperse dyes (Disperse Red 153, Disperse Yellow 211, Disperse Blue 56, and a combination of the three to make black) were used to dye samples of polyester fabric. Fibers from each sample were cut into 1mm pieces to represent trace evidence that could be found at a crime scene. Extraction of the dyes was done using an existing standard operating procedure and subsequently prepared for analysis.

APPI is a type of gas phase ionization technique used with MS. However, most studies done with dyes have focused on other, more common ionization techniques, such as ESI. Disperse dyes, because of their chemical nature, can be ionized using APPI, whereas some other dye types, like reactive dyes, cannot be seen using APPI. This study takes advantage of this feature to compare the sensitivity of APPI to ESI coupled with a quadrupole Time-Of-Flight Mass Spectrometer (qTOF-MS) under the same electronic conditions for the detection of disperse dyes extracted from fibers.

While commercial dyes are typically used in the textile industry, they come with certain drawbacks when it comes to analysis. These dyes are often patent protected, and the structures are not always correctly given. Two dyes may have the same Color Index (CI) value but different structures. In addition, commercial dyes are often not pure. This creates difficulties when trying to distinguish impurities from degradation products, which can be important when examining trace evidence. To address these problems, three similar disperse dyes (red, yellow, and blue) from North Carolina State University's historic Max Weaver Dye Library were chosen from the database using open-source Konstanz Information Miner (KNIME) software with a Tanimoto coefficient greater than 90%. This database contains 2,196 dyes that are unique to the Max Weaver Dye Library. Since the structures of these dyes are known, fragmentation spectra obtained using Tandem Mass Spectrometry (MS/MS) can be compared to those of the commercial dyes and help to predict possible dye structure similarities and degradation products.

Results show that the sample size required for detection using APPI is significantly less than that when using ESI for both commercial and Max Weaver disperse dyes extracted from trace fibers. This finding shows that APPI enhances the sensitivity of the dye characterization by MS.

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### Mass Spectrometry, Atmospheric Pressure Ionization, Dyes

## D4 An Analysis of Polymer Composition and Dye of Textile Fibers by Direct Analysis in Real Time-Mass Spectrometry (DART<sup>®</sup>-MS) and Raman Microscopy

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**Learning Overview:** After attending this presentation, attendees will better understand the capabilities of the DART<sup>®</sup>-MS and Raman microscopy for identifying the unique combination of polymer and dye constituting the fibers found at crime scenes. The pros and cons of both techniques, their complementary characteristics, and the possibility of combining these methods with other instrumental techniques will be presented.

**Impact Statement:** This research will impact the forensic science community by introducing quick and effective methods to textile fiber analysis that will aid in the advancement of the field. Besides enhancing the specificity of fiber identification based on the analysis of the unique combination of polymer and dye, both the DART<sup>®</sup>-MS and Raman methods have integrated the process of microextraction of analytes for successful dye identification in fibers weighing only 0.44-0.56mg.

The Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART<sup>®</sup>-MS) and the Surface-Enhanced Raman scattering (SERS) method were developed to analyze dyes on the textile fibers in this study. Past studies have illustrated that the TD-DART<sup>®</sup>-MS can be used for the successful identification of the polymeric backbone structures of different textile materials, such as cotton, nylon, polyester, cellulose triacetate, poly(propylene) and poly(acrylonitrile). The TDP/DART<sup>®</sup>-MS method also yielded other chemical information, such as the dyes.<sup>1</sup>

Currently, there are thousands of textile dyes either derived from natural sources or synthetic pathways. Dyes are often classified into different categories according to their unique application method and chemical composition.<sup>2</sup> This classification, and the effectiveness of correctly identifying dyes of similar colors, hold high importance in connecting a suspect to trace evidence, such as carpet or clothing fibers.<sup>3</sup>

This study focused on nine blue dyes from five categories, including acid, basic, disperse, and blue/violet, commonly used in manufactured textiles. A TD-DART<sup>®</sup>-MS method was developed to analyze dyes and polymeric materials on an array of applicable fibers, which included silk, cotton, and nylon. The mass spectra were collected from the dye standards and dyes applied to fibers, with no extraction techniques. The multidimensional profile, including both the desorption time and mass spectra, was compared. The characteristic ions of dyes and polymeric materials were identified.

Dispersive Raman spectrometers with both 532nm and 785nm laser excitation were also used for the fiber analysis. Aqueous silver colloid was added to the fibers prior to microwave-assisted dye extraction to improve the signal-to-noise ratio of the Raman spectra of dyes while minimizing the contribution of background signal due to the polymer. The novel microwave-assisted dye extraction method was carried out in capillary tubing containing the dyed fiber and silver colloid derived from the Leopold and Lendl method. The subsequent analysis of dye adsorbed onto silver colloids showed a significant amplification of the Raman signal along with a distinct decrease of spectral interference due to fluorescence from the textile dyes or polymers.<sup>4</sup> The unique Raman bands of the extracted dyes were distinguishable among the blue dyes studied.

The TDP/DART<sup>®</sup>-MS method requires a single fiber sample size and sample preparation time of approximately two minutes, but it offers a multidimensional profile within approximately seven minutes. Due to the method being both simple to perform and having high sample throughput potential, this method may significantly contribute to the identification of dyes on fibers at crime scenes.

The Raman data are based upon the project supported by the National Science Foundation under Grant No. 1920293. The MTSU Undergraduate Research Experience and Creative Activity (URECA) program is acknowledged for its support for this study.

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### Fiber, DART<sup>®</sup>-MS, Raman Microscopy

## D5 A Comparison of Weathering Profiles of Ignitable Liquids (ILs) by Gas Chromatography/Mass Spectrometry (GC/MS) and Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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**Learning Overview:** Attendees will better understand the weathered chemical profiles of various ILs using GC/MS and DART®-MS.

**Impact Statement:** This presentation will impact the scientific community by showing the effect of weathering processes on the spectral profiles of the ILs and a comparative study of weathered samples on GC/MS and DART®-MS.

In many arson cases, fire is initiated by employing ILs.<sup>1</sup> Therefore, in the arson investigation process, the identification of ILs and IL residues at the scene plays a key role in identifying the cause of the fire and distinguishing the ILs used to initiate the fire. The most commonly used technique to analyze the fire debris is GC/MS; however, it has a major limitation of analyzing only the volatile components in ILs. The non-volatile or less-volatile components in ILs are likely to be contained in the fire debris and, hence, could yield corroborating evidence on the use of specific ILs in the investigation. DART®-MS is a sensitive tool with excellent analytical sensitivity to analyze non-volatile components in ILs.<sup>2</sup> In this study, the IL weathered chemical profiles are compared by GC/MS and DART®-MS methods.

In the present study, four ILs were selected: gasoline, diesel, kerosene, and Japan Drier. Aliquots of 1–10mL of each IL were weathered at three different temperatures, 30°C, 90°C, and 210°C, to different degrees of weathering at 30–99% mass reduction of IL prior to being analyzed. To analyze the IL samples on DART®-MS, an automated sample introduction apparatus with Linear Rail Enclosure that holds consumable Quickstrip™ sample cards were used. A 5µL sample volume was spotted on the Quickstrip™ card after diluting the sample in chloroform. For the GC/MS analysis, 20µL of the sample was added to 1mL of chloroform, followed by an injection of 1µL with a split ratio of 1:50.

The DART®-MS data were analyzed by averaging the mass spectra of ~60 scans after the spectral background subtraction. The mass spectral profiles of gasoline and Japan Drier are relatively consistent irrespective of weathering percentage. In the case of diesel and kerosene, as the weathering percentage increased, the mass spectral patterns shifted toward the higher mass range. The Total Ion Chromatogram (TIC) of GC/MS data of all ILs has shown a distinctive pattern corresponding to the extent of weathering. Collectively, for the highly weathered IL samples, the relative peak intensities increased for the less-volatile compounds. To evaluate the experimental factors in the sample preparation, such as weathering percentages and weathering temperatures, Analysis Of Variance-Principal Component Analysis (ANOVA-PCA) was used. The data indicated that the weathering percentage is the major factor responsible for the variance of the TIC data. The GC/MS data suggested that it is challenging to correlate the weathered IL to the non-weathered IL, because the weathering process significantly alters the relative quantities of the IL. The DART®-MS data of ILs provided less variable profiles and therefore may not be used to predict the weathering percentage of ILs. However, the capability of DART®-MS in detecting the less volatile IL constituents at high m/z values imparts its unique ability to discriminate among different types of ILs. Examples of these non-volatile additives include polyisobutylene succinimides and polyether amines in gasoline and carboxylate salts of zirconium, manganese, and cobalt in Japan Drier.

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### Ignitable Liquids, Weathering, DART®-MS



## D6 Validation Testing of a Syntactic Authorship Identification Method Using Ground Truth Data From 20 Authors

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**Learning Overview:** In this presentation, attendees will learn about validation testing in forensic linguistic evidence, specifically author identification, in order to meet the reliability and error rate criteria for scientific evidence.

**Impact Statement:** This presentation will impact the forensic science community by providing results of validation testing using the Syntax-Based Authorship Identification (SynAID) method to determine the reliability and error rate of computational forensic linguistic author identification.

The first validation testing of forensic authorship identification methods came quite late to the field. Svartik coined the term “forensic linguistics” and used a syntactic and statistical analysis to determine authorship in a case.<sup>1</sup> However, afterward, no testing of the method to determine its reliability or error rate was performed. In fact, for close to three decades, academic linguists were providing reports and sometimes even testimony about authorship using methods that had never been tested to determine their reliability or accuracy.

Chaski began testing authorship identification techniques on “ground-truth” data (documents whose authorship was known), independent of any litigation, in order to determine error rate.<sup>2,3</sup> For most of the tests, Chaski used four authors who were matched by age, dialect, educational level, and gender so any differences in style could not be attributed to demographics. Chaski’s results demonstrated that most ideas about authorship identification were highly unreliable, and even a combination of these techniques would get no better than 50% accuracy. Chaski published a validation test of a technique based on syntactic analysis, using text analysis software to analyze the syntactic patterns, syntactically classified punctuation and word lengths of texts to produce a numerical profile that is statistically analyzed using a leave-one-out, cross-validated discriminant function analysis (the SynAID method).<sup>4</sup> In this validation testing, Chaski used ground-truth data from ten authors who were matched for age, dialect, and educational level with five men and five women.

Using the same dataset, Hirst and Feiguina performed validation testing of a different syntactic analysis method, the frequency of syntactic labels over two words in a row.<sup>5</sup> Chaski reported additional validation tests.<sup>6</sup> Nini and Grant performed validation testing using ground-truth data from three authors and, using non-syntactic features, could not differentiate those three authors.<sup>7</sup> Juola reports a validation test of his software using blog posts, which are probably not ground truth data due to the nature of the internet and blog writing conventions.<sup>8</sup>

In the validation test in this presentation, ground-truth writing of 20 authors who have similar demographics are subjected to the SynAID method. The 20 authors are all male, White, middle-aged, college-educated, native English speakers with some executive or managerial experience. Their writing samples were all spontaneously written during the course of their daily lives. Test results with error rate and accuracy rate are reported for over 100 documents from these 20 authors. The 20 authors’ document numerical profiles were subjected to pair-wise statistical analysis using the protocol described in Chaski.<sup>4</sup>

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### Validation, Authorship Identification, Ground Truth Data

## D7 Examining Conceptual Burstiness and Its Discursive Purpose in Violent Extremism Cases

*Awni Etaywe\**, University of New South Wales, Sydney, New South Wales, Australia

**Learning Overview:** This presentation showcases how to explore concepts with burstiness in discourse and how an empirical language analysis of “conceptual burstiness” can help security and intelligence investigators understand terrorist texts and interpret crucial information and semiotic cues on terrorism cases.

**Impact Statement:** This presentation provides cues applicable for risk contexts to suspect, characterize, and understand terrorism cases, particularly cues on intentions, agendas, ideological grounds for violence, and radical ideological perceptions. These linguistic contributions are a crucial part of what prosecution and intelligence analysts can use; failure to consider such contributions to evidence interpretation means that threat assessment and prosecution can easily go awry.

Linguistics can help security, intelligence, and law enforcement agents understand terrorism cases.<sup>1</sup> To date, limited forensic linguistic research has been undertaken on conceptual burstiness as a discursive marker in transnational terrorism cases despite recognition of the usefulness of this language feature in text authorship tasks, marking genres, topics and authors, and information retrieval.<sup>2,3</sup> This presentation showcases how an empirical language analysis of conceptual burstiness can help investigators understand terrorist texts and interpret crucial information on terrorists’ agendas, ideologies, affiliations and stances, and on the discursive purpose of drawing on particular concepts. Repetition of particular concepts is taken as characteristic of the burstiness of these concepts and their subcategories. This presentation explores a set of “bursty concepts,” as textual elements of violent extremism, in a corpus of 20 terrorist public statements communicated openly (via the internet and media outlets) over 2001–2019 by four transnational terrorists: former al-Qaeda leader Osama bin Laden; former ISIS leader al-Baghdadi; Boko Haram leader Shekau; and the far-rightist Brenton Tarrant.

To elevate accuracy and reduce subjectivity, the most frequent lemmas were initially explored by AntConc software (version 3.5.8.0).<sup>4</sup> To enable a broad overview of these lemmas, the number of times of word occurrence was set to a minimum of 10. For accuracy purposes, counts of synonyms such as *messenger* and *prophet*, *brother* and *brethren*, *Allah* and *God/lord* (in e.g., Shekau’s texts), or *iman* (Arabic word) and *faith* were added to each other, making one lexeme. The resulting lists of lemmas have produced a list totaling 8,659 repetitions of 264 lemmas, that is, 21.65% of the entire specialized corpus. The semantic categories of the groups of lexis were annotated manually. Based on a close examination of the lemmas in their concordance lines, the running concepts were identified. That is, words with close semantic proximity—whether encoding related concepts, indexing or evoking particular intertextual worlds (e.g., economics, religion, etc.), belonging to specific semantic fields or domains of experience (e.g., the military), or groupable under certain lexical sets identifiable in Roget’s Thesaurus—were grouped together under superordinate and subordinate groups of concepts, such as “violence” and “violent action,” respectively. The patterns of the most frequent lemmas were compared with full-fledged corpus linguistics studies of lexical categories of extremist discourse. Findings showed a network of bursty concepts of 5 superordinate categories—“people,” “religion,” “power,” “violence and military,” and “sayers” categories—and 17 subcategories. The concepts served as indicators of elements such as the underpinning religious and ethnic ideologies, the ideological motivations of violence threatened or incited for, the terrorists’ agendas of violent actions, their geographical spread, terrorists’ interests in political and economic control over out-groups, the participants involved, and their acts of stance taking.

Burstiness in the dataset was found as a function of the discursive purpose to which the heightened use of concepts is mobilized in these texts. That is, the terrorist texts tended to drive the burstiness of identified lemmas and their conceptual categories to serve the terrorists’ purposes, such as presenting the terrorists as powerful social actors in the argumentative discourse of violence, generating fear, prescribing specific forms of violence, legitimization of violence, and provoking hostility against religiously or politically different “others.”

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### Extremism, Forensic Linguistics, Transnational Terrorism

## D8 Validation Testing for Authorship Identification Outside and Inside Casework

Carole E. Chaski, PhD\*, Institute for Linguistic Evidence, Georgetown, DE

**Learning Overview:** After attending this presentation, attendees will better understand how validation testing occurs in the Syntax-Based Authorship Identification (SynAID) method for forensic author identification, both outside and inside casework.

**Impact Statement:** This presentation will impact the forensic science community by providing an example of how validation testing can occur within each case as well as outside casework, a technique that could be helpful to other methods of pattern-based evidence.

The first validation testing of forensic authorship identification methods began in 1997 with testing of authorship identification techniques on “ground-truth” data (documents whose authorship was known), independent of any litigation, in order to determine error rate.<sup>1,2</sup> Most of these tests used four authors who were all native speakers of English, matched by age, dialect, educational level, and gender (i.e., a group controlled by demographics, which influence language use). Validation test results demonstrated that most ideas about authorship identification were highly unreliable, and even a combination of these techniques would get no better than 50% accuracy, a result that was replicated by other scholars.<sup>3</sup> Again, conducted outside casework, a validation test of a technique based on syntactic analysis, using text analysis software to analyze the syntactic patterns, syntactically classified punctuation, and word lengths of texts to produce a numerical profile that is statistically analyzed using a leave-one-out, cross-validated discriminant function analysis (the SynAID method) was published.<sup>4</sup> In this validation testing, ground-truth data from ten authors, who were matched for age, dialect, and educational level with five men and five women was used. The SynAID method was able to differentiate the documents of ten authors and assign the documents to the correct author with 95% cross-validated accuracy. Using the same dataset, Hirst and Feiguina validated a different syntactic analysis method, the frequency of syntactic labels over two words in a row.<sup>5</sup> Furthermore, additional validation tests of the SynAID method was reported.<sup>6</sup> Validation tests of the SynAID method outside casework continue and are crucial to meeting current reliability standards for forensic science.<sup>7-10</sup>

One difference between the SynAID method and other methods of forensic author identification is that the SynAID method provides a validation test of the method *inside each case*. The SynAID method first determines how well the known suspects can be differentiated from each other, creating a statistical model of authorship, then, using leave-one-out cross-validation tests, the statistical model of authorship to see how many times each known document will be assigned to the correct author. Thus, for each case in which SynAID is used, the SynAID method is tested on known documents (i.e., a case-internal validation test is conducted). If the method can differentiate and assign known documents with high accuracy (over 80%), then and only then are the questioned documents assigned statistically to one of the suspects. This study presents the results of over 20 such case-internal validation tests. Most importantly, the SynAID method demonstrates that other methods that rely on pattern-based evidence could implement the same technique of validating the method on known items in the case itself before handling questioned items.

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### Forensic Authorship Identification, Validation Testing, Pattern Evidence

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## D9 Algorithmic Fairness: Sampling and Algorithmic Bias Correction in Machine Learning With Applications to Forensic Evidence

*Ganesh Krishnan, PhD\**, Iowa State University, Ames, IA and Center for Statistics and Applications in Forensic Evidence, Ames, IA; *Heike Hofmann, PhD*, Iowa State University, Ames, IA and Center for Statistics and Applications in Forensic Evidence, Ames, IA

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**Learning Overview:** Attendees will learn about fairness in machine learning algorithms and their impact on automated forensic evidence pattern matching

**Impact Statement:** This presentation brings to the forefront a major issue with algorithmic pattern matching, addresses fairness concerns, and provides a unique way to get unbiased and reliable results

Machine learning algorithms fundamentally rely on the training sample being representative of the population under consideration. This becomes an issue when dealing with new samples that are considered not from the same population, or when the training sample is not a random sample of the underlying population. In such cases, the predictions from machine learning algorithms can lead to incorrect results. In usual settings, it is not possible to identify what the true underlying population is, and this becomes even more complicated when dealing with high-dimensional settings. Therefore, the training sample may or may not represent the true nature of the data that is to be modeled. This can lead to incorrect predictions and misclassified results. When dealing with critical areas like forensics, such incorrect predictions can have far-reaching effects and influence court proceedings catastrophically.

One such case arises when the class of new samples is from a multivariate location-shifted distribution when compared to the training sample distribution. When dealing with classification performance using probabilistic scores from machine learning, and under specified conditions, this problem can alternatively be viewed as the inability to get equal error rates at a pre-specified classification threshold when the test data set (or new unknown samples) and training samples are different in terms of the location parameter. With the goal of removing bias that stems directly from such covariate shifts, and ensuring equal error rates, a robust method of transforming the original problem of classification to a problem of classification via dependence modeling is provided for a class of scale-invariant machine learning algorithms. This is done in a way that the new predicted outcome is now considered “fair” with respect to sampling bias and algorithmic bias stemming from covariate shifts. Both sampling and algorithmic bias from covariate shifts can be treated differently depending on the algorithm under consideration. However, it is shown that when using random forests under the proposed conditions, sampling and algorithmic bias from covariate shifts are two sides of the same coin.

The performance of the proposed method was evaluated for forensics ballistics data. For the case of bullet matching algorithms, it can be specifically seen that the problem of covariate shifts, or multivariate location-shift, is analogous to training and test data sets representing different firearms. It is then shown that the proposed method outperforms previous state-of-the-art methods in automatic bullet matching in situations of shifted covariates.<sup>1</sup> Thus, robust predictions results are provided, agnostic to the firearm under consideration. The proposed method is also agnostic to the nature of forensic evidence data as long as the assumptions of method are satisfied and data quality standards are maintained to ensure that the sampling and algorithmic bias are only from covariate shifts.

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### Pattern Matching, Covariate Shifts, Firearm Examination

## D10 Validation Testing of the SynAID Method for Authorship Identification Using Ground Truth Data from Four Non-Native English Authors

Ángela Almela, PhD\*, Universidad de Murcia, Murcia, Spain and Institute for Linguistic Evidence, Georgetown, DE; Pascual Cantos Gómez, PhD, Universidad de Murcia, Murcia, Spain

**Learning Overview:** Attendees will better understand validation testing in forensic linguistic evidence, specifically author identification, using the SynAID method on a dataset of non-native English speakers to meet the reliability and error rate criteria for scientific evidence.

**Impact Statement:** This presentation will have an impact on the forensic science community by providing validation testing results using the SynAID method to determine the reliability and error rate of computational forensic linguistic author identification when non-native English speakers write the documents.

The first validation testing of forensic authorship identification methods occurred quite late to the field. Svartvik coined the term “forensic linguistics” and used syntactic and statistical analysis to determine authorship in a case.<sup>1</sup> However, no testing of the method to determine its reliability or error rate was done after that. Indeed, for nearly three decades, academic linguists have been providing reports and, in some cases, testimony about authorship using methods that have never been tested to determine their reliability or accuracy. Chaski began testing authorship identification techniques on “ground-truth” data (documents whose authorship was known), independent of any litigation, in order to determine error rate.<sup>2,3</sup> For most of the tests, Chaski used four authors who were all native speakers of English, matched by age, dialect, educational level, and gender, so that any differences in style could not be attributed to sociolects. Chaski’s results demonstrated that most ideas about authorship identification were highly unreliable, and even a combination of these techniques would get no better than 50% accuracy. Chaski published a validation test of a technique based on syntactic analysis, using text analysis software to analyze the syntactic patterns, syntactically classified punctuation, and word lengths of texts to produce a numerical profile that is statistically analyzed using a leave-one-out, cross-validated discriminant function analysis (the SynAID method).<sup>4</sup> In this validation testing, Chaski used ground-truth data from ten authors, who were matched for age, dialect, and educational level with five men and five women. Using the same dataset, Hirst and Feiguina validated a different syntactic analysis method, the frequency of syntactic labels over two words in a row.<sup>5</sup> Furthermore, Chaski reported additional validation tests.<sup>6</sup>

In the validation test presented, the ground-truth writing of four authors who were highly educated, sophisticated authors, and non-native speakers of English is analyzed. The known documents are subjected to the SynAID method, and validation test results with an error rate and accuracy rate are reported.

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### Forensic Computational Linguistics, Authorship Identification, Syntax

## D11 A Comparison of Two Computational Approaches to Analyzing Last Words

*Cristina Aggazzotti, PhD, Institute for Linguistic Evidence, Georgetown, DE*

**Learning Overview:** After attending this presentation, attendees will have learned about two computational approaches for classifying the last words of inmates on death row.

**Impact Statement:** This presentation will impact the forensic science community by discussing and comparing two computational forensic text classification approaches on a relatively understudied text genre.

Most natural language processing research on final statements before death have focused on suicide notes with the aim of automatically identifying suicidal thoughts in order to help prevent a subsequent suicide. The last words of inmates on death row have primarily been treated as a comparative dataset for testing suicide note classifiers rather than as the focus of the classification research.

Recent work has shown that various versions of Long Short-Term Memory (LSTM) models are becoming more accurate for suicide note classification.<sup>1-3</sup> One follow-up study expanded on this work by additionally implementing a Transformer Recurrent Neural Network (TransformerRNN) to classify suicide notes, last words, and neutral online blog posts.<sup>4</sup> The study found that although the TransformerRNN achieves new state-of-the-art in overall performance and in classifying suicide notes, LSTMs with attention perform better at classifying last words specifically.<sup>1,5</sup>

ALIAS Suicide Note Assessment REsearch (SNARE), a computational tool that implements Linear Discriminant Function Analysis (LDFA) with leave-one-out cross-validation, achieves competitive accuracy on the similar task of distinguishing real suicide notes from various other related text genres, including simulated suicide notes, apology letters, love letters, and angry letters, among others.<sup>6</sup> A previous pilot study applied SNARE to a dataset of last words to determine which text genres last words most closely resemble as a means of shedding light on the mental state of inmates immediately prior to death.<sup>7</sup>

Based on the recent success of LSTMs with attention for classifying last words, the present study implements an LSTM within SNARE to analyze the datasets of last words, suicide notes, and the various control document genres. The results of this preliminary test will be presented and compared to the LDFA results on the same task. Implications of this experimental comparison and directions for future research will also be discussed.

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### Computational Forensic Linguistics, Natural Language Processing, Last Words

## D12 The Lineup Technique for Forensic Author Identification

*Carole E. Chaski, PhD, Institute for Linguistic Evidence, Georgetown, DE*

**Learning Overview:** After attending this presentation, attendees will have learned how the lineup technique can be used properly in forensic author identification.

**Impact Statement:** This presentation will impact the forensic science community by providing principles of the lineup technique in forensic author identification when only one suspect related to the case investigation is available, a technique that can be extended to other kinds of document examination.

The “lineup technique” is used for eyewitness and earwitness identification of a suspect in relation to distractors. Currently known as the “sequential” lineup, the eyewitness is performing a pair-wise comparison between the photograph and the memory.<sup>1-4</sup> In an earwitness lineup, voice samples are presented sequentially.<sup>5</sup> Underpinning all lineup techniques is the fundamental fairness of the procedure. A fair lineup is one in which the suspect and the distractors are similar enough that the suspect does not stand out from the distractors unfairly.

The lineup technique can be used for authorship identification under certain conditions. First, the lineup technique should only be used when there is only one suspect in the case. If there are several possible authors of a questioned document, all of the suspects should be tested in the “candidate pool.” There is no need for a lineup. Second, the distractors must be similar to the suspect. For authorship, the distractor authors must have the same demographics that affect language as the suspect author. The lineup authors must match the suspect author’s native language, sex, age range, education, race, work experience, and contemporaneous. It is patently unfair, although it has happened in case reports, to compare a 21<sup>st</sup>-century Black American prisoner to 20<sup>th</sup>-century French literary authors or to compare a 21<sup>st</sup>-century native-Swedish law professor to 19<sup>th</sup>-century native-English literary authors. Such unfairness should immediately disqualify experts who are obviously trying to rig their methods to identify the Black American prisoner or exculpate the Swedish law professor, just as any court would disqualify a lineup in which a male suspect is compared to only female distractors. Third, the lineup should follow the sequential, pair-wise testing to determine if the suspect author and each lineup author can be differentiated, and, if so, to whom the questioned document belongs.

This presentation presents an example of the lineup technique in which the SynAID method of forensic authorship identification was deployed. A sheriff was accused of harassing and cyberstalking a citizen by producing 11 anonymous documents. First, prosecutorial investigators had no other suspect than the sheriff. A lineup method was warranted. Second, the sheriff’s demographics were known so that 19 distractors who matched his demographics were used in the lineup. The 19 distractors were all contemporaneous, native-English, White, middle-aged, college-educated men with some executive experience. Third, 19 pairwise tests were run sequentially. Each line-up author was tested against the suspect to determine how well the SynAID method could differentiate the known writings of each author. If the statistical model of known authorship is over 80% accurate, then the model is used to assign each questioned document independently to one of the pair. The average accuracy of the 19 models of authorship was 95%. Given these strong models of authorship, the assignment of the questioned documents then occurred. Each of the 19 models of authorship overwhelmingly identified the sheriff rather than the distractor as the author of each questioned document. Finally, a binomial probability is used to determine the likelihood that a questioned document will be assigned  $x$  out of  $y$  tests.

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### Forensic Author Identification, Lineup Technique, Syntax

## D13 A Comparison of Authorship Identification Methods Using Ground Truth Data from Four Non-Native English Authors

*Pascual Cantos Gómez, PhD\*, Universidad de Murcia, Murcia, Spain; Angela Almela, PhD, Universidad de Murcia, Murcia, Spain and Institute for Linguistic Evidence, Georgetown, DE*

**Learning Overview:** After attending this presentation, attendees will better understand validation testing in forensic linguistic evidence, specifically authorship identification, using three different methods on the same dataset to meet the reliability and error rate criteria for scientific evidence.

**Impact Statement:** This presentation will impact the forensic science community by providing validation testing results to determine the reliability and error rate of using three different methods of author identification when the documents are written by non-native English speakers.

In the field of forensic sciences, the testing of methods to determine their reliability or accuracy prior to their application to cases has proved to be of utmost importance. However, in forensic linguistics, further proper validation testing of authorship identification methods is needed. The first syntactic and statistical analysis to determine authorship in a case was presented in 1968.<sup>1</sup> Still, no testing of Svartvik's method was performed to assess its reliability or error rate afterward. In fact, for close to three decades, academic linguists have been providing reports and sometimes even testimony about authorship using methods that have never been tested to determine their reliability or accuracy.

Chaski began testing authorship identification techniques on "ground-truth" data (documents whose authorship was known), independent of any litigation, to determine error rate.<sup>2,3</sup> For most of the tests, Chaski used four authors who were all native speakers of English, matched by age, dialect, educational level, and gender, so that any differences in style could not be attributed to demographics. Chaski's results demonstrated that most ideas about authorship identification were highly unreliable, and even a combination of these techniques would get no better than 50% accuracy. Chaski published a validation test of a technique based on syntactic analysis (the SynAID method).<sup>4</sup> In this validation testing, Chaski used ground-truth data from ten authors, who were matched for age, dialect, and educational level with five men and five women. Using the same dataset, Hirst and Feiguina performed validation testing of a different syntactic analysis method.<sup>5</sup> Chaski reported additional validation tests in her law review on best practices.<sup>6</sup> Nini and Grant performed validation testing using ground-truth data from three authors and, using non-syntactic features, could not differentiate those three authors.<sup>7</sup> Stamatatos presented a validation test of a character-based method, using texts that were all the same genre and topic.<sup>8</sup> However, matching topic and genre does not reflect the reality of forensic casework. Juola reports a validation test of his software using blog posts.<sup>9</sup> However, blog posts are probably not ground truth data due to the nature of the internet and blog writing conventions.

Generally, the three approaches to author identification are syntax-based, word-based, or character-based. These approaches can be tested using software called SynAID, LexiAIDE, and UniAIDE. In the validation test in this presentation, ground-truth writing of four authors who were highly educated, sophisticated authors, and non-native speakers of English, writing on different topics and in different but comparable genres, is analyzed. The known documents are subjected to the SynAID, LexiAIDE, and UniAIDE methods that measure syntax, words, and Unicode characters, respectively. The validation tests determine how often the method correctly identifies the author of each document in the dataset. Validation test results with an error rate and accuracy rate are reported for the syntactic, character, and lexical approaches to meet the reliability and error rate criteria for scientific evidence.

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### Forensic Computational Linguistics, Authorship Identification, Validation Testing



## D14 A Quantitative Comparison of Last Words to Related Text Genres

Cristina Aggazzotti, PhD, Institute for Linguistic Evidence, Georgetown, DE

**Learning Overview:** After attending this presentation, attendees will better understand the last words of inmates on death row as a text genre and will learn about the characteristics they share with other related genres.

**Impact Statement:** This presentation will impact the forensic science community by quantitatively analyzing last words and identifying their similarities and differences with related text genres to which they have not yet been compared.

Previous research has either focused on last words as its own text genre or compared them to suicide notes and depressed notes.<sup>1-5</sup> This present study expands upon this research by examining last words in relation to not only suicide notes, but also several other text genres, such as apology letters and trauma narratives.

This project is an extension of a previous pilot study that uses ALIAS Suicide Note Assessment REsearch (SNARE), a computational tool that employs linear discriminant function analysis with leave-one-out cross-validation. SNARE compares a questioned document to a database composed of over 400 real suicide notes and 500 control documents ranging over several text types: apology letters, love letters, trauma narratives, angry letters, complaint letters, simulated and real threats to known targets, business letters related to insurance, and simulated and hoax suicide notes.<sup>6</sup> The output of SNARE is a quantitative classification of the questioned document as either a real suicide note or a control document.

SNARE obtains an accuracy of 80% distinguishing real suicide notes from control documents and an even higher accuracy of 86% for suicide notes limited to 45 words or less.<sup>6</sup> In comparison, mental health professionals obtain an accuracy of 63% to 71% identifying suicide notes as real or not.<sup>7,8</sup>

The original pilot study tested a subset of the data—the first 100 final statements of death row inmates in Texas starting in 1982—which is publicly available on the Texas Department of Criminal Justice website.<sup>9</sup> The results showed that last words were more often classified as a suicide note (85 out of 100) than as a control document (15 out of 100).

The present study extends the pilot study by incorporating all of the data (445 total final statements) and digging deeper into the results to examine the features that last words share with the comparison text genres. Viewing last words through the lens of each of these genres can help improve SNARE as a suicide note classifier and can provide further insight on the perspectives held by inmates immediately preceding death.

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### Computational Forensic Linguistics, Last Words, Suicide Note

## D15 Critical Flow Behavior in Forensic Engineering Accidents

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**Learning Overview:** After attending this presentation, attendees will understand the mechanism underlying forensic engineering accidents caused by critical flow behavior.

**Impact Statement:** This presentation will impact the forensic science community by introducing the importance of critical flow behavior in forensic engineering accidents.

If a working fluid, such as compressible gas, is expanded across a flow device (e.g., a nozzle, an orifice, a valve, or the end of a pipe exit), its velocity and specific volume changes with upstream or downstream pressure. As the inlet pressure increases or the outlet pressure decreases, the gas velocity through the flow device reaches a sonic condition. When the gas velocity reaches sonic velocity, further increases in upstream pressure do not cause any further increases in gas velocity through the flow device. Once the sonic velocity is reached, further increase in the upstream pressure causes an increase of density, which in turn results in an increase of the mass flow rate. In the case of a fixed inlet condition, further decreases in the downstream pressure do not cause any further increase of either the velocity or the mass flow rate. These phenomena play key roles in pressure-relieving devices for vessels and an ignition mechanism in flammable gas system, such as an oxygen-enriched system.

This presentation introduces one case of an explosion accident that happened in a plant at a valve exit of an oxygen-enriched system. It is reported that fires in oxygen-enriched systems require a source of energy to trigger ignition. The most common ignition energy sources are mechanical impact, particle impact, friction, and pneumatic impact or compression heating.<sup>1</sup> When high purity oxygen under high pressure is released quickly through a valve into a low-pressure environment, the velocity can reach a sonic condition, resulting in near-adiabatic compression at an outlet flow obstacle. This phenomenon was verified through an experiment using nitrogen gas. The experiment was simulated by Computational Fluid Dynamics (CFD) with a change in the working fluid from nitrogen to pure oxygen.

Experiments with one spring-loaded pressure relief valve and one rupture-disk pressure relief valve with increasing inlet pressure at a fixed ambient exit pressure are also presented. The volumetric flow rate does not increase when the absolute pressure ratio reaches 1.89, which corresponds to the sonic velocity condition. The mass flow rate increases linearly regardless of the sonic velocity condition but remains almost constant above the absolute pressure ratio of 22.

This study introduces an analysis tool for the critical flow behavior of gas that can cause plant-related accidents and the flow characteristics of specific pressure relief valves in accordance with the pressure ratio that can explain the failure mechanism of a pressure vessel.

**Reference(s):**

- <sup>1</sup> American Society for Testing and Materials. Standard Guide for Control of Hazards and Risks in Oxygen Enriched Systems. ASTM 1995; G128-95.

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### Forensic Engineering, Critical Flow, Pressure-Relief Device

## D16 A Visualization of Overlapping Fingerprints by Hyperspectral Imaging in the Visible Region

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of a non-destructive detection method of fingerprints with a hyperspectral imager and a separation method of overlapping fingerprints.

**Impact Statement:** This presentation will impact the forensic community by providing a non-contact, non-destructive, and non-invasive method to detect fingerprints with a hyperspectral imager and a laser. It is shown that overlapping fingerprints can be effectively utilized by this method.

Fingerprints are one of the clues of personal identification and are important evidence in criminal investigations.<sup>1</sup> Fingerprint identification is done by comparing their minutiae. However, in past cases of the two or more overlapping fingerprints, even if the minutiae were found out, the overlapping part was not available for identification because it is not clear which fingerprint the minutiae of the overlapped part belonged to. Since the components of the fingerprint depend on the individual, the fluorescent spectra of each fingerprint are different and also change over time.<sup>2</sup> Therefore, if the fingerprint can be imaged using its different spectral information, it is possible to separate it into a single fingerprint.

Hyperspectral Imaging (HSI) is a method of obtaining a spectrum for each pixel in an image of the entire scene. It was reported that overlapping fingerprints were separated by subtracting and dividing the images acquired by HIS.<sup>3</sup> Furthermore, Principal Component Regression (PCR) allowed this study to separate the overlapping fingerprints more clearly.<sup>4</sup> In this study, Multivariate Curve Resolution-Alternating Least-Squares (MCR-ALS) was used to separate overlapping fingerprints in order to make better use of overlapping fingerprints.

Two overlapping fingerprints were prepared. The images of the fingerprints were acquired with a hyperspectral imager in a wavelength range of 414.7-983.6nm. The resolution of wavelength was 1.0nm, and the resolution of image was 600 x 960 pixel; 532nm continuous-wave laser was used as a light source.

Fluorescence spectra of the two fingerprints were observed from 560 to 760nm, and were different from each other. First, PCR was performed using 200 images from 560 to 760nm. As a result, two single fingerprint images were obtained in up to the sixth principal component. These images were clearer than those by subtraction and division methods. However, unclear parts in the fingerprints remained. One of the reasons is that loadings of PCR are permitted negative, although fluorescence spectrum is ordinarily only positive. On the other hand, since the calculation in the MCR-ALS is carried out under a non-negative constraint condition, it is possible to resolve into the spectrum similar to the actual spectrum. Since MCR-ALS requires initial conditions, it was calculated using the PCR components as initial conditions. As a result of spectral resolution by MCR-ALS and PCR, the areas that were unclear by the method of only PCR became clearer. It was demonstrated that the MCR-ALS and PCR method using HSI for the separation of overlapping fingerprints could be considered more effective.

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### Fingerprint, Hyperspectral Imaging, Fluorescence

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## D17 A Semi-Permeable Membrane Device for Canine Explosive-Detection: A Rate of Evaporation Study

*Madison Reavis, BS\*, IUPUI, Indianapolis, IN; John Goodpaster, PhD, IUPUI, Indianapolis, IN*

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**Learning Overview:** Attendees of this presentation will gain information and understanding about the quantity of odor that emits from a Semi-Permeable Membrane Device (SPMD) and its applicability to canine explosive-detection training.

**Impact Statement:** This presentation will impact the forensic science community by presenting information about the training of canines for explosive-detection using an SPMD for odor delivery and training aid storage.

When not in use, training aids used for canine explosive-detection training are typically stored in ammo cans held in large magazines, although there is no generally accepted storage method. Due to the lack of a perfect seal in the ammo can, explosive volatiles often contaminate materials that are stored nearby. To ensure canines are trained using uncontaminated training aids, an SPMD is being tested as a new way to store training aids to eliminate contamination and to train canines via odor delivery.

The SMPDs being tested consist of a glass jar, a membrane holder, and a gas-tight lid. The membrane holder, housed between the jar and gas-tight lid, contains an odor permeable membrane that is sealed by a gasket.

The goal of this research is to quantify the amount of explosive odor that escapes from the SPMD compared to unconfined explosives to determine if SPMDs can be used for canine training. The data obtained from this study will determine if evaporation rates of common solvents from the SPMD are predictable, reliable, and reproducible from day-to-day and from SPMD to SPMD.

Data for this research was obtained using a high-precision analytical balance. For each experiment, the SPMD was filled with 30mL of solvent and the membrane holder and gas-tight lid were then sealed. The gas-tight lid was removed before the SPMD was placed on the balance. The mass of the SPMD and its contents were recorded every 60 seconds for 60 minutes. This data was plotted to generate a graph of mass loss as a function of time. Data regression was performed to obtain values used to calculate evaporation rate relative to n-butyl acetate, which has a known and standardized rate of evaporation.

Preliminary data suggests that there is an exponential relationship between relative boiling point and evaporation rate. It has been determined that compounds with higher boiling points have lower evaporation rates than compounds that have lower boiling points, such as r-limonene (BP 178°C) and nitromethane (BP 101°C) with relative evaporation rates of 0.07 and 3.57, respectively. Work is currently being done to further explore this relationship and to determine an equation that dictates it. The materials studied thus far include nitromethane, nitroethane, 1-nitropropane, 2-ethyl-1-hexanol, r-limonene, Dyno Unimax, 2,4-dinitrotoluene, and tetryl. The effect of pore size in the odor permeable membrane on evaporation rate is also currently being investigated.

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**Forensic Explosive Analysis, Canine Training Aids, Semi-Permeable Membrane Device**

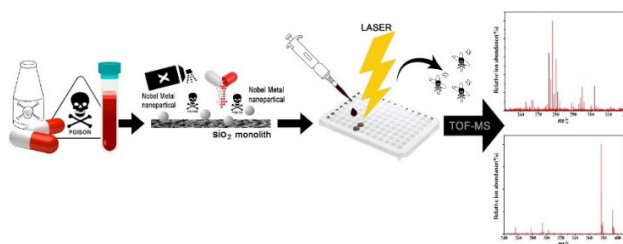
## D18 Noble Metal-Decorated Silica Monoliths for Forensic Analysis of Drugs and Pesticides Using Mass Spectrometry (MS)

Entesar Al-Hetlani, PhD\*, Kuwait University, Khaldyia, Asimah, Kuwait; Mariam Ismail, MSc, Kuwait University, Khaldyia, Asimah, Kuwait; Mohamed Amin, MSc, Kuwait University, Khaldyia, Asimah, Kuwait

**Learning Overview:** The use of a minute amount of sample is of great interest to forensic scientists; most evidence recovered in the crime scene is of limited quality and quantity, which can affect its analysis. This work demonstrates the use of a minute amount of sample; drugs, pesticides, drugs in blood serum, and pesticides in beverages to detect species of forensic interest. The use of Surface Assisted Laser Desorption/Ionization-Mass Spectrometry (SALDI-MS) has recently proven to be a very powerful technique and highly suitable for forensic applications due to the relatively simple requirements and small sample consumption. It has been used for fingerprint, explosive, drug, and textile fiber analyses and produced satisfactory results with a good limit of detection. Drugs have always been one of the most commonly encountered evidence; recently, pesticides and rodenticide have emerged as a major concern for forensic scientists. As demonstrated by Blakey et al., such species have also been used as adulterants in street drugs.<sup>1</sup> Therefore, analysis of drugs and pesticides using SALDI-MS has a great potential for narcotics and toxicology applications.

**Impact Statement:** Recently, research into forensic drugs and pesticides analyses has gained wide attention. This is motivated by the rapidly developing street drug market and emergence of new drugs mixed with several adulterants. Several researchers have focused on drugs in biological fluids using conventional chromatographic techniques coupled with extraction for sample cleanup. Therefore, crime scene investigation of traces in a beverage or biological stain and the presence of drugs and poisons is of great significance. Therefore, this study explored the analysis of beverage and blood serum spiked with drugs and pesticides, respectively, using Surface-Assisted Laser Desorption/Ionization-Mass Spectrometry (SALDI-MS) using a small amount ( $\approx 2\mu\text{L}$ ). The obtained mass spectra were investigated in terms of the effect of sample deposition and the presence of electrolytes and were carefully studied. Using Au-SiO<sub>2</sub> as a substrate offered the best results, with a Limit of Detection (LOD) of 10pg/mL to 1fg/mL for drugs and 10 g/mL to 10fg/mL for pesticides. Despite the presence of salts at high concentrations, the detection of drugs and pesticides was feasible. Future applications of the developed substrates can be extended to the analysis of Performance-Enhancing Drugs (PEDs) used by professional athletes.

Over the years, the development of MS methods has generated limitless possibilities for forensic toxicology and drugs analyses, where qualitative and quantitative analysis of drugs and poisons continue to be one of the most concerning issues to forensic investigators. Drugs are substances mainly used to cure diseases; however, they can be abused and be the source of violence, crimes, or even death. Furthermore, fatal poisoning by pesticides is not uncommon because there are cheap to obtain, widely available and accessible, and, therefore, can be used in suicide cases. In this work, four noble metal nanoparticles (NPs) Au, Ag, Pt, and Pd (M) were introduced on silica-based (SiO<sub>2</sub>) monoliths 3D skeleton (M-SiO<sub>2</sub>) via chemical reduction. Subsequently, they were used as SALDI-MS substrates for drugs and pesticides detection. The loading amount of the M-NPs on SiO<sub>2</sub> monolith, reducing agent concentration and reaction time, were optimized for all the substrates and monitored using an Ultraviolet/Visible (UV/Vis) spectrophotometer. Elemental and morphological analysis proved the successful loading of spherical noble metal NPs on SiO<sub>2</sub> monoliths without affecting the original morphology of the SiO<sub>2</sub> monoliths. The M-SiO<sub>2</sub> substrates were then employed for SALDI-MS; the results revealed that the sample deposition method influenced the SALDI performance and Au-SiO<sub>2</sub> substrate demonstrated the best results compared to other substrates in terms of the signal-to-noise (S/N) ratio. In addition, the tolerance toward electrolytes was investigated and Au-SiO<sub>2</sub> substrate exhibited high salt tolerance and detection was possible even at 500mM of NaCl. Finally, non-biological and biological samples, particularly carbonated malt drink and blood serum spiked with drugs and pesticides, respectively, were analyzed using Au-SiO<sub>2</sub> substrate. The prepared substrate offered sensitive detection of the analytes with a Limit Of Detection (LOD) of drug-spiked drinks of 1ng/mL for doxepin, 100ng/mL for desipramine, 1μg/mL for imipramine, amitriptyline, and trimipramine and pesticide-spiked serum of 100ng/mL for dimethametryn and thiodicarb, and 1ng/mL for metalaxyl and napropamide and a relative standard deviation (%RSD) of 7.21-22.22% and 2.68-26.29% for drugs and pesticides, respectively. There was no sample preparation for the carbonated drink and very simple preparation for the blood serum, yet the analytes were identified with low LOD and high shot-to-shot reproducibility. Au-SiO<sub>2</sub> monolith proved to be an efficient tool to detect small molecules in limited amounts of sample and thus their forensic applications could be further expanded.



**Figure 1.** Schematic illustration of the experiments employed for the determination of drugs in blood serum and pesticides in spiked carbonated beverage. A 2μL amount of the sample was deposited on the optimized Au-SiO<sub>2</sub> monolith and analyzed using SALDI-MS to obtain the relative mass spectra for the drugs and pesticides.

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### Forensic Drugs and Poison Analysis, Beverage and Blood Serum, Mass Spectrometry

## D19 Automation in Forensic Science: The Future Ahead

*Rattandeep Kaur, Msc\*, Panjab University, Chandigarh, India; Surya Kant Tripathi, PhD, Panjab University, Chandigarh, India*

**Learning Overview:** The goal of this presentation is to help attendees understand the challenges that are confronted in the application of Artificial Intelligence (AI) and Machine Learning (ML) to the field of forensic science.

**Impact Statement:** This study will impact the forensic science community by presenting the challenges faced by a forensic scientist while deploying AI and ML tools for the analysis of evidence and data. The probable solutions for the problems will be presented so that the benefits of AI and ML can be garnered by the forensic science community.

Technology has been an elixir for human beings and has grown and evolved just like humans due to which the role of humans has slowly diminished. AI and ML have proven their potential application in tasks like the Automated Fingerprinting Identification System, Forensic Ballistics Identification System, Probabilistic Genotyping Algorithms, etc. However, there is still wariness in accepting these automated systems openly for forensic applications as has been done in other fields. One of the reasons for this is that of all of the other areas, criminal justice and forensic science are the areas where the transition to automated systems is looked at with extreme caution.

The focus of this study is to present the problems caused by the introduction of automated systems in forensic case evaluations. The computational algorithms are found to be opaque, which makes them difficult to discern or control. This in turn leads to inefficient ML process and such uncertainties and errors in turn affect the output of the systems. The inefficiency in automated systems can be a result of the opaque nature of the algorithm. This opaqueness can be intentional, technical, or inherent. On one hand where market domination and competition are the reasons for intentional opacity, lack of skills and the presence of complex systems are the causes for technical and inherent opacity, respectively.<sup>1</sup> Probabilistic genotyping constitutes the use of statistical methods and computing in order to perform DNA profiling and has been one such area where the foundational validity of the methods used is sometimes challenged.

On the solution part, reviewing source code is one of the solutions posited, and efforts should be made to critically and scientifically evaluate the output of such automated systems.<sup>2</sup> As suggested in the National Academy of Sciences Report in 2009, research must be done with the intention of understanding the reliability and repeatability of the comparison methods used. Efforts should be made to design an algorithm that is comprehensible. Forensic scientists should make sure that the automated system they choose is capable of addressing the problem concerned and, last but not least, automated systems should be able to explain the decision-making process to the users.<sup>2</sup>

In summary, misrepresentation of facts by the automated systems can lead to jeopardizing human rights; hence, efforts should be made to align the evaluation of evidence on such protocols that are able to strengthen the trust in forensic science and eventually in the criminal justice system.

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**Artificial Intelligence, Algorithms, Forensic**

## D20 Physical Evidence Demonstrating Poor Maintenance in the Cause of an Axle Fracture and a Rollover Crash With Injuries

*Kurt Weiss, MSME\*, Case Study Collision Science, LLC, Santa Barbara, CA; Mark Pozzi, MSSE, Sandia Safety Science, Edgewood, NM; David Bosch, PhD, Forensic Engineering, Inc., Phoenix, AZ*

**Learning Overview:** Forensic investigators will become informed as to how and why catastrophic axle failures occur, and the related circumstantial and physical evidence that can be analyzed to properly investigate such incidents.

**Impact Statement:** The forensic community will benefit from learning about these relatively uncommon but predictable failures that often result in catastrophic injury and death. The loss of control, traffic collisions, and rollover events typically occur at highway speeds with little or no warning. Forensic investigators involving criminalistics, engineering sciences, and jurisprudence should have particular interest in this topic.

At approximately 60mph, the driver of a small domestic SUV suddenly felt like something broke, and he could no longer control his vehicle. The vehicle unexpectedly steered left into the center median, where the right-side tires furrowed in the dirt, precipitating a passenger-side leading rollover. The right rear wheel assembly that had completely separated from the SUV was located during an inspection of the incident site. Although they had been replaced only three days before the crash, it was concluded the tires did not cause or contribute to the collision.

The SUV was inspected, and the entire rear axle assembly was removed to allow for high-resolution microscope and borescope imaging of the axle shaft. Approximately one cup of differential oil was drained in the process. The exposed end of the fractured axle shaft revealed the entire end bearing was missing, and the axle housing showed an unexpected wear pattern (Figure 1). Additionally, there were telltale signs of friction-generated heat on the axle housing adjacent to the failed end (Figure 2).



Located inboard relative to the flange assembly and wheel lug nuts, the solid axle features a bearing surface about which the end bearing rotates (Figure 3). Compared to the left axle shaft, the failed right component demonstrated a bearing surface with artifacts of excessive wear, abrasions, and marking related to metal-to-metal contact (Figure 4). It was concluded that insufficient lubrication contributed to the bearing failure. The failure likely occurred due to a lack of maintenance and inadequate inspection of the differential, pinion gear housing, and axle bearings prior to the incident.



Vehicle axle failures are uncommon, but are typically caused by lack of axle or bearing lubrication; failure to replace cotter pins in castle nuts leading to failure of hubs; overloading; roadway-induced impacts, collision-related damage; or in-service failures due to inadequate materials and design. The latter type of failure can cause an axle to fail catastrophically with little or no warning, often with no damage to the tire or wheel prior to axle fracture. The resulting loss of control often leads to collision or rollover.

Fuel economy and cost-reduction initiatives of the 1970s promoted downsized light trucks and SUVs, wherein passenger car axles are used to save weight and cost. Additional cost reductions eliminated pressed-on inner bearing races by converting the axle shaft surface into the inner bearing race. This process involved machining a bearing race into the axle and induction heating to harden the outer axle surface so it could withstand bearing wear. This had the negative effect of significantly reducing the diameter of ductile tough steel as the outer axle shaft surface was converted to brittle hardened steel. The net effect was axles that were not as durable or safe for their predictable use in trucks and SUVs on rough roads, towing, or hauling heavier loads. This led to increased incidents of operational failures of such axles in non-collision circumstances.

The dangers of heat-weakened axle shafts are exacerbated when they also require straightening, often as a direct result of the heat hardening of the inner bearing race area. The axle shaft is placed in an induction heater and straightened while red hot. This process is often repeated as necessary, sometimes as many as four times. This additional heating often further embrittled the outer surface of the axle, creating even greater likelihood of failure.

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## **Axle Failure, Bearing Race, Rollover Crash**



## D21 The Mechanics of All-Terrain Vehicle (ATV) Rollovers

*David Pienkowski, PhD\*, University of Kentucky, Lexington, KY*

**Learning Overview:** Attendees will gain a new appreciation for the risks of sideway and backward rollovers of youth and adult ATVs and the contributions to these risks attributable to varying terrain angles, throttle inputs, and number of ATV riders.

**Impact Statement:** Information provided in this presentation will aid the forensic engineer investigating the mechanics of ATV accidents because of the new data quantifying: (1) terrain angles at which backward rollovers become possible, especially when a second rider is positioned behind the ATV driver, and how this possibility can further increase due to maximum ATV throttle input; (2) relationships between ATV speeds and turn radii on level and terrain of varying incline angles; and (3) differences in sideway and backward rollover between youth- and adult-sized ATVs.

ATVs have enabled new mobility for those in rugged country terrain, but these vehicles are also associated with considerable injuries and deaths. ATV rollover is an unappreciated injury mechanism responsible for significant morbidity and mortality. Rollovers account for 63% of ATV crashes, making them the most prevalent type of ATV injury mechanism. Youths are more likely than adults to roll sideways on flat terrain, but adults are more likely to roll backward while riding uphill. ATV rollover injuries frequently occur because the hazards of ATV use are unrecognized or under-appreciated. The objective of this study was to quantify the mechanics attending side or backward rollovers for youth- and adult-sized ATVs for single and dual riders on varying terrain inclinations with varying engine throttle inputs.

Two new exemplar ATVs (one youth-sized, one adult-sized) were tested in a variety of side- and rear-directed inclinations in a controlled static laboratory environment using mathematical simulation. Centers of gravity of these ATVs were measured for one adult or one adult and one child rider (located at various seating positions on the ATV) for actual angular inclinations and simulated engine throttle positions. Rollover was considered to occur when the center of gravity extended beyond the footprint of the studied ATV tires.

The analyses showed that youth-sized ATVs are slightly less susceptible to sideway rollover than adult-sized ATVs. The presence of a rider behind the ATV driver, however, altered these findings. Thus, while youth-sized ATVs are slightly less susceptible to sideway rollover than adult-sized ATVs for a single rider, they are more susceptible to sideway rollover than adult-sized ATVs when a rider accompanies the driver.

The minimum turn radius that an ATV can safely negotiate increases sharply with increasing ATV speed. This is especially evident for speed changes from low initial take-off velocities. Specifically, an ATV can navigate a 3-4 four-foot turn radius only at low (10fps) speeds, while doubling the speed of the ATV to 20fps increases the safe minimum turn radius approximately 5–6-fold.

The likelihood of ATV tip-over with sharp radius turns is substantially increased by inclined terrain. A mild and commonly encountered 20° decline changes the minimum safe turn radius for an ATV moving at 30fps from approximately 35 feet to approximately 58 feet with a single occupant (the driver). A rider sitting behind the ATV driver doubles this safe minimum turn radius.

Backward rollovers become more likely as forward-approaching terrain inclination angles exceed 40° for youth- and adult-sized vehicles. This angle is substantially reduced in the adult-sized ATV when maximum throttle input is applied and to a lesser extent for the lower horsepower youth-sized ATV. The likelihood of backward ATV rollover also increases with the presence of a second rider behind the driver. Maximum throttle and a second rider elevate the likelihood of backward rollovers and reduce the critical angles of forward-approaching terrain angles to 15°–21°. Although youth-sized ATVs are almost equally susceptible to static backward rollover when only the driver is present, youth-sized ATVs are less tolerant of a rider behind the driver than adult-sized ATVs.

Risks inherent to actual ATV rollovers prohibited empirical testing and limited methods to static laboratory experimentation and theoretical torque values based on estimated engine responses. In conclusion, risk of sideway and backward rollover of ATVs can occur while turning at unexpectedly low speeds, especially when performed on inclined terrain, when full throttle is applied, or extra riders accompany the driver on the ATV. Compared to adult-sized ATVs, youth-sized ATVs do not provide substantial additional protection from sideway or backward rollovers.

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### ATV Accident, ATV Rollover, ATV Injury

## D22 Runaway Mustang

*Michael Rosenfield BS\*, RSR Engineering, LLC, Mesa, AZ; Robert Anderson, MS, Biomechanics Analysis, Tempe, AZ*

**Learning Overview:** Through a case study, attendees will be shown the steps taken to collect relevant physical and electronic evidence that was used to arrive at the conclusion of the existence of a sudden unintended acceleration that resulted in a significant automobile crash.

**Impact Statement:** This presentation will impact the forensic science community by presenting a case study that provides an example of an actual sudden unintended acceleration as well as outlining the investigation and analysis that was used to arrive at the conclusion that a sudden unintended acceleration had, in fact, occurred.

In this angled, head-on, rollover crash, a convertible driven by a teenager with three other teenage passengers ran a stop sign and struck a pickup. The convertible's driver claimed that a brake failure had prevented the vehicle from stopping. The passengers stated they heard the driver yelling, "I can't stop!"

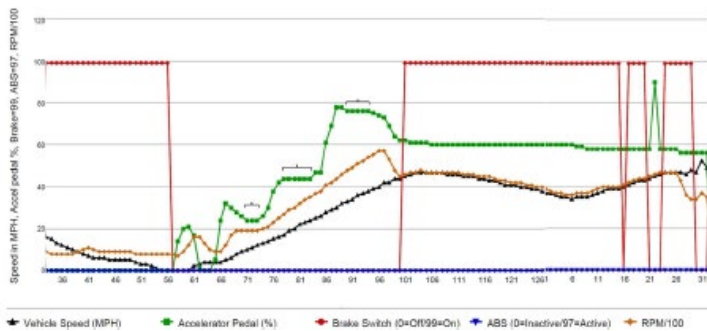


After failing to stop the vehicle at stop sign at a T-intersection, the driver reportedly switched strategies and attempted to make a right turn onto the main road but went into oncoming traffic. The assignment was to inspect the brake system to determine if there had been a brake failure and obtain pre-crash accelerator pedal and brake data from the Crash Data Retrieval (CDR) report to evaluate whether or not the driver had a pedal error.

Post-crash inspection of the convertible's brake system revealed that although the plastic master cylinder reservoir had become detached from the master cylinder during the collision, the hydraulic system was otherwise undamaged and in working order.

During the vehicle inspection, the Powertrain Control Module (PCM) was found to have significant damage, with a broken circuit board and missing components. Fortunately, the memory chip was still present so that the PCM data was obtained after a chip swap onto a surrogate PCM board.

The PCM data showed that the convertible accelerated at steady 1/4g, while the accelerator was applied in a stepwise fashion for 1,000 feet before crashing into the pickup at about 50mph. This distance from the stop to the crash matches the vehicle's described path. The constant 60-percent accelerator pedal, with and without brake application, is inconsistent with a pedal error, but could indicate a stuck accelerator pedal.



The convertible was re-inspected, which showed that the accelerator pedal was entrapped in the top groove of the floor mat. In addition, the mat had longitudinal scrapes from the bottom the accelerator pedal and transverse impressions where the pedal appeared to have been held at discrete locations, including at the edges of the oval depressions.



This case study serves as an example of how a comprehensive look at the available evidence may be required to "get it right." The combination of the physical evidence, electronic evidence, and witness statements leave little doubt that there was an entrapped accelerator pedal that led to a runaway vehicle situation. Choosing to ignore or not pursue additional available evidence is a gamble that could lead to an incorrect conclusion. This case shows the importance of approaching an investigation with an open mind and looking for facts before jumping to conclusions.

### Sudden Unintended Acceleration, Chip Swap, Floor Mat

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## D23 Recreation Vehicle (RV) Crashworthiness: Enhanced Injury Caused by Unsafe Vehicle Interior Structures Combined With Predictable Seat and Belt Failures

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Vijay Gupta, PhD, University of California-Los Angeles, Los Angeles, California; Jack Bish, PhD, SAFE Laboratories, Goleta, CA*

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**Learning Overview:** This presentation shows a forensic field investigation that has been conducted to identify the cause and effect of these dangerous designs, as well as static and dynamic testing that discovered the failure mode of the seats and belts, as well as the predictable extreme hazards of unpadding interior structures. Safer alternative seat, head, and belt restraint designs were discovered that would have prevented hazardous occupant contact with the unpadding force-concentrating structures.

**Impact Statement:** This presentation will benefit the forensic community and humanity by showing that these severe hazards exist in RVs, why they exist, how they create enhanced injury, and how those injuries can be prevented using lightweight, economically and technologically feasible means. Vehicle crash investigators will learn how to identify recreational vehicle seat and belt failure, vehicle interior occupant contact witness marks, and associated forensic evidence that will allow more accurate assessment of occupant injuries in such circumstances.

Seat and belt failure in rear impact has been known to cause severe injury and death to both front and rear seat occupants. Unpadding rigid interior structures located within predictable occupant contact zones exacerbates these hazards. A case of predictable enhanced head injury was discovered in a recreational vehicle where a multi-position seat and lap-only belt failure was combined with rigid, unpadding, force-concentrating structures in the vehicle occupant compartment. Causation of these enhanced injuries was proven by a combination of accident reconstruction, human surrogate testing, and dynamic sled testing. Prevention of injury by lightweight, technologically and economically feasible means was also demonstrated.

It has been known for decades that head impact into narrow, force-concentrating structures can result in skull fracture, brain injury, spinal injury, and death at much lower load levels than if those same structures were adequately padded, contoured, or otherwise modified to reduce their lethality. To reduce or eliminate such injuries, Federal Motor Vehicle Safety Standard (FMVSS) 201, Occupant Protection from Interior Impact, has been part of the Code of Federal Regulations related to vehicle crashworthiness since 1967, with updates in the 1990s. It has required certain interior structures within the range of occupant contact zones to be effectively padded, flattened, rounded, and otherwise made less lethal when contacted by vehicle occupants, whether belted or unbelted. Therefore, dash panels and other vehicle interior trim is padded, and narrow, projecting controls and other structures have been eliminated in the forward areas of passenger vehicles for decades. These requirements are not applicable to the rear occupant seating areas of RVs, however, due to loopholes in the safety regulations.

Several contradictions to the well-known foregoing occupant protection principles have been discovered in late-model recreational vehicles, in which unpadding, force-concentrating structures have been placed into the predictable path of vehicle occupant's heads and upper torsos that are allowed to catapult rearward or sideways because of predictable seat and belt design deficiencies and failures. RV safety regulations permit lap-only belts and do not require head restraints for anything other than the front outboard seats. They also allow side-facing seating while the vehicle is in operation. These dangerous conditions have resulted in enhanced catastrophic injuries to vehicle occupants that would otherwise not have occurred if the seat had been in a forward-facing position, was crashworthy enough to remain reasonably upright, was equipped with an effective head restraint, and had an effective belt restraint system. Unless the seat remains upright, the belts and head restraints are not effective at protecting the occupant. The absence of energy-absorbing padding and the multitude of rigid, force-concentrating structures in the rear of the recreational vehicle interior exposes occupants to these extreme hazards even while wearing the ineffective lap-only belts while seated in low-back seats.

The reason that these dangerous conditions are occurring in late-model recreational vehicles is that there are no effective occupant protection standards for such vehicles, especially because there are no dynamic seat or belt performance standards for rear impact, and there are no requirements for equivalent padded structures in the rear of vehicle interiors like those found in the front-seating areas per FMVSS 201.

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### RV Crashworthiness, Seat and Belt Failure, Unsafe Vehicle Interior

## D24 Heavy Truck Fuel Tank Protection in Frontal Impacts

Brian Herbst, BS\*, SAFE, Goleta, CA; Steven E. Meyer, PE, SAFE, Goleta, CA; Jack Bish, PhD, SAFE Laboratories, Goleta, CA; Lauren Bell, BS, SAFE, Goleta, CA; Christopher Clarke, BS, SAFE, Goleta, CA; Chuck Rogers, SAFE, Goleta, CA

**Learning Overview:** This presentation explores three real-world crashes wherein a heavy truck experienced a frontal offset impact with a resulting fire and serious injury.

**Impact Statement:** This presentation will impact engineers, scientists, attorneys, police, and others involved in the analysis of vehicle accidents, specifically heavy truck crashes resulting in fire.

The dangers of exposed heavy truck front suspension and steering components and the risk of underrun in frontal collisions were recognized decades ago.<sup>1</sup> Since the 1970s, industry literature and government studies noted the dangers of heavy truck frontal crashes, suggested improving safety in these accidents, strengthening front suspension components and adding protective structures. The United States Department of Transportation (DOT) published Truck Occupant Protection in 1986, found post-crash fires were involved in 16% of heavy truck fatalities compared to 4% for cars.<sup>2</sup> The report identified several mechanisms of fuel tank rupture, including frontal impacts resulting in front axle contact with fuel tanks. In order to ensure vehicle occupants who survive a crash are not subjected to a post-crash fire, the fuel system should be designed for crash conditions an occupant is expected to survive.

The hazards of exposed side saddle fuel tanks have been known for decades, including hazards specific to heavy truck tanks mounted outside the frame rails.<sup>3-6</sup> This fuel tank arrangement was abandoned decades ago in light vehicles due to its vulnerabilities. Heavy trucks still use the outboard tank location despite the known hazard.

In 1994, the United Nations Economic Commission for Europe (ECE) published a standard for heavy truck Front Underrun Protective Structures (FUPS), which became the basis for a similar Australian standard adopted in 2009. However, the United States still has no requirements regarding front underrun protection of heavy trucks. A FUPS prevents underride and engages the energy-absorbing structures of smaller impacting vehicles, provides protection of the truck's steering components, and helps prevent the truck's front axle from being displaced into the fuel tank, which can cause rupture and fire.

Three real-world crashes are presented wherein a heavy truck experienced a frontal impact, resulting in fire and serious injury. In each of these cases, sled testing was conducted on a production truck front structural assembly and compared to a similar FUPS equipped assembly. The effectiveness of FUPS in mitigating damage in these frontal crashes was assessed.

**Case Study 1:** The right side of a heavy truck side swiped the left rear of a parked tow truck, resulting in a flash fire. The production sled testing impacted the truck assembly at 27.4mph, induced significant damage, and broke away the front axle. The FUPS-equipped test was impacted at the same speed, but with significantly reduced penetration and depth of damage into the truck's structure. The FUPS increases the initial stiffness and energy-absorption characteristics of the front structure, allowing for earlier and faster separation of the accident vehicles and thereby reducing the probability of fuel tank interaction.

**Case Study 2:** A heavy truck impacted a bear in the roadway with its left front bumper, causing tire deflation and loss of control. The truck continued off the roadway, interacted with roadside terrain, and caught fire. The sled testing impacted an exemplar truck front assembly with a 255lb bear surrogate at 60mph and demonstrated significant interaction with and rotation of the steering wheel. This interaction would affect the driver's ability to maintain steering control. The sled testing showed that a FUPS virtually eliminates wheel interaction, preserving steering control.

**Case Study 3:** An SUV crossed the center line and impacted a heavy truck, causing major damage to the left front of both vehicles. The impact compromised the truck's fuel tank and caused a large explosion. Full-scale testing showed that a properly constructed FUPS was able to effectively deflect a mid-sized SUV at 55mph, while protecting the front axle and fuel tank.

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4. "Heavy Vehicle Crash Injury—A Survey." Henderson and Sims, Traffic Accident Research Unit, Department of Motor Transport, New South Wales, January 1970.
5. "Heavy Truck Fuel System Safety Study." DOT HS 807 484, NHTSA Technical Report, September 1989.
6. Heavy Trucks—Fatal Accident Reporting System Special Report on Heavy Trucks, M. Cassidy, NHTSA, May 1978.

### Heavy Truck, Fuel Tank, Protection

## D25 Unintended Recliner Connecting Rod Actuation in Rear Impacts: A Forensic Analysis

Steven E. Meyer, PE, SAFE, Goleta, CA; Brian R. Herbst, PhD, SAFE, Goleta, CA; Jeremy McMillin\*, SAFE, Goleta, CA; Arin Nelson, SAFE, Goleta, CA; Davis Hock, SAFE, Goleta, CA; Jack Bish, PhD, SAFE Laboratories, Goleta, CA

**Learning Overview:** This presentation explores three real-world crashes wherein automotive seats have exhibited catastrophic failure due to recliner release via the recliner release rod, undermining the seat's ability to safely restrain the seated occupant and causing serious injury in rear-impact automotive collisions.

**Impact Statement:** This presentation will impact engineers, scientists, attorneys, police, and others involved in the analysis of vehicle restraint systems, specifically rear-end automotive crashes wherein front outboard seats are found excessively reclined post-crash.

In rear-impact automotive collisions, occupant safety, for both front- and rear-seated passengers, relies on free-standing seats to effectively restrain their seated occupants without intruding into the survival space of other occupants in the vehicle.<sup>1-3</sup> Restraining the occupant heavily depends on the seat's ability to keep the seatback sufficiently upright as to not allow the occupant to ramp up and out of the seatback before absorbing crash energy and providing effective occupant ride down. Occupant ramping can present harmful interactions with vehicle structures and/or other occupants, particularly children, seated in the vehicle.

Three real-world crashes will be presented wherein forensic analysis of post rear-impact crash evidence established recliner release during the rear-end collision event via actuation from the recliner release rod. This unintended recliner actuation caused/contributed to catastrophic seat back collapse, undermining the seat's ability to absorb crash energy and contain the occupant.

**Case Study 1:** A Hyundai® Accent® sedan was slowing for traffic when it was rear-ended by a Ford® Windstar® mini-van. The driver's seat of the Hyundai® collapsed rearward, and the driver ramped up and out of the seat, impacting rear structures of the vehicle, resulting in traumatic head injuries. Forensic analysis of the seat revealed the inboard side of the seat tore below the recliner, rotated the recliner release rod, and released the outboard recliner. No significant structural load deformation, evidencing load transfer, was found on the outboard seat structure.

**Case Study 2:** A Nissan® Titan® pick-up truck was stopped at a red light when it was rear ended by a Toyota® Sequoia® SUV. The driver's seat of the Nissan® collapsed rearward, allowing the driver to ramp up and out of the seat, impact rear structures of the vehicle, and resulted in cervical and thoracic spine fractures. Forensic analysis of both front seats revealed asymmetrical deformation below the inboard and outboard recliner, which resulted in rotation of the recliner release rod and recliner release on the lesser deformed side of the seat.

**Case Study 3:** A Honda® Fit® hatchback was slowing for stopped traffic when it was rear ended by a Chrysler® 300 sedan. The driver's seat of the Honda® failed to remain upright, allowing the driver to ramp up and out of his seat, and make forcible head contact with his son seated in the rear seat, resulting in skull fractures and traumatic brain injury to the child. Analysis of the seat structural deformation, when compared to the crash energy, indicated significantly less plastic deformation than expected based upon the structure's documented yield strength. Forensic analysis of the driver's seat revealed occupant interaction with the recliner release rod bent, creating a rotational moment at the connecting rod, and released both recliners.

Laboratory testing was utilized in each case to establish the recliner release via rotation of the recliner release rod.

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3. J. Saczalski; A. Sances, Jr.; S. Kumaresan; J.L. Burton; P.R. Lewis, Jr. Experimental Injury Study of Children Seated Behind Collapsing Front Seats in Rear Impacts. Paper No. 2003-0046, 0067-8856/2003, Published in the *ISA Proceedings on Biomedical Sciences Instrumentation*, Vol. #39, pp 259 265, 2003.

### Crashworthiness, Rear Impact, Seat Back

## **D26 Event Data Recorder (EDR) Data and Forensic Video Analysis Defends Municipality in Civil Rights Lawsuit After Officer Forcefully Subdues Fleeing Driving Under the Influence (DUI) Suspect**

*Kurt Weiss, MSME\*, Case Study Collision Science, LLC, Santa Barbara, CA; Parris, Ward, JD, Biodynamics Engineering, Inc., Pacific Palisades, CA*

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**Learning Overview:** This presentation will show how careful review and analysis of case evidence led to overlooked information vital to a municipality's defense of a police use-of-force case.

**Impact Statement:** This presentation will demonstrate the traffic reconstruction that uses a multidisciplinary approach of combining roadway geometry, EDR pre-crash and crash data, along with the analysis of audio and video evidence that revealed facts that contradicted the plaintiff's sworn testimony.

Excessive speed and erratic vehicle movements alerted a law enforcement officer to what he suspected was an impaired driver. Without a turn indicator, the suspect suddenly exited the highway, and at the bottom of the off-ramp, unexpectedly accelerated in an attempt to flee. The officer gave chase, and both vehicles sped dangerously through a maze of residential streets. After descending a steep decline, the subject's pickup truck appeared to become airborne while crossing an intersecting road and proceeded the wrong way down a one-way street. The subject then failed to negotiate a sharp turn and his vehicle impacted a curb, striking several large sandstone boulders. The impact caused the frontal airbags to deploy, and knocked the large boulders into the roadway. The pickup was disabled and coasted to a stop, giving the chasing officer time to attempt apprehension.

The patrol car's onboard forward-facing video camera captured the incident. The subject exited his disabled pickup and appeared to have difficulty standing. The officer ordered the subject to "get down on the ground." The subject initially sat on the ground, but then stood up and attempted to flee by stumbling up a ramp to a business. The officer ran toward the subject and attempted to subdue him, and a struggle ensued. At one point, the officer pulled the subject up to a standing position, pivoted, and threw the subject down on the ramp. A second officer arrived on scene, and the subject was taken into custody and transported to a local hospital because he complained of back pain.

The subject suffered an L1 vertebral body compression fracture requiring spinal fusion at T11-L3 and lumbar laminectomy at T12-L2. The toxicological report revealed the presence of amphetamine and methamphetamine. The incident prompted a lawsuit alleging the officer's excessive force caused the spinal injury. At deposition, the subject testified that his back broke when he was slammed to the ground, describing pain like being hit with a sledgehammer.

Seventeen months after the incident, the subject's vehicle was located in a body shop, and arrangements were made to image the EDR. Pre-crash data provided crucial insight into the vehicle's speed prior to the curb strike. The incident scene was photographed, measured, and laser scanned. The collision reconstruction combined the vehicle's pre-crash speed data with the onboard video. The analysis showed the approximate vertical acceleration experienced by the subject when the vehicle slammed down after being airborne was the likely source of his spinal injury.

Audio and video evidence from two patrol cars also supported this defense. Both officers were wearing body-worn microphones that connected to their in-car recording systems. The first officer's microphone malfunctioned and that of the second officer recorded only loud music playing in his patrol car that obscured what could be heard on video. It was believed that conversations between the officers and subject were not recorded. However, when the second officer's audio channel was isolated, and the in-car videos were synchronized and enhanced, the mechanism of injury could be supported, and the subject's comments to officers could be heard. Immediately after the fall, the subject did not cry out in pain. Rather, he asked officers to let him go and said, "I hurt my back in that accident." The case was resolved in settlement before trial.

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### **EDR, Compression Fracture, Audio/Video Evidence**

## D27 A Method for the Evaluation of Recreational Off-Highway Vehicle (ROV) Rollover Protective Structures (ROPS)

*Brian Herbst, BS\*, SAFE, Goleta, CA; Christopher Clarke, BS, SAFE, Goleta, CA*

**Learning Overview:** After attending this presentation, attendees will better understand the history of the ROPS design standards used for ROVs and a ROPS testing methodology that is well suited to simulating real-world rollover conditions.

**Impact Statement:** This presentation will impact the forensic community by exploring an ROV rollover case study and presenting a testing methodology for evaluating ROV ROPS performance.

In recent decades, there has been significant development in the ROV sector and manufacturers now offer ROVs with unprecedented performance. Unfortunately, this new class of vehicles also presents unprecedented dangers.

Rollover has long been known as a significant cause of injuries and fatalities in off-highway vehicles. The **Society of Automotive Engineers** (SAE) published its first recommended practice for ROPS design in 1967, intended to protect earthmoving equipment operators traveling less than 10mph in limited operating conditions.<sup>1</sup> The SAE-recommended practice relied on force and energy absorption requirements in quasi-static loading tests, and, by 1980, it had been adopted as the **International Organization for Standardization** (ISO) 3471 standard.<sup>2</sup> The Occupational Safety and Health Administration (OSHA) adopted another SAE quasi-static test method for agricultural tractor ROPS structures in the 1970s.<sup>3</sup>

The Recreational Off-Highway Vehicle Association (ROHVA) adopted the ISO and the OSHA ROPS testing requirements in the 2011 edition of its American National Standards Institute (ANSI) -ROHVA standard.<sup>4</sup> Unfortunately, these decades-old standards were intended for slow vehicles like bulldozers and have little relevance to the dynamic loading conditions that can occur in rollover accidents involving high-speed modern ROVs designed for sport driving on rough terrain. The ISO and OSHA tests generate minimal intrusion in the ROV ROPS, while real-world rollovers can result in the collapse of ANSI-ROHVA-compliant ROPS.

A rollover accident was evaluated in which a ROV traversed a sand dune at a speed of 25–30mph, became airborne, pitched nose down, and completed one roll end-for-end, coming to rest on its wheels. This type of rollover scenario would be unimaginable for a bulldozer but is not uncommon in high-performance ROVs due to their short wheelbase, long suspension travel, and sport driving capabilities. During the subject rollover, the ROPS interacted with the ground and crushed down into the occupant space. One of the vehicle occupants was paralyzed. Although the ROPS had been designed to comply with the ISO and OSHA ROPS standards, it failed catastrophically under the conditions of the real-world rollover. A number of other field accidents involving that same ROV exhibited a similar pattern of ROPS failures.

Drop testing is a standard impact test method utilized routinely to evaluate structural integrity and impact resistance in a number of different industries. Various entities have run inverted drop tests from as high as 3 to 12 feet in order to evaluate vehicle rollover performance. Inverted drop testing was conducted to evaluate the subject ROPS design as part of the subject accident investigation. An exemplar vehicle was inverted and suspended 30 inches above a load plate, then released to fall onto its ROPS under the influence of gravity. The test generated structural failures in the ROPS and intrusion into the occupant space consistent with what occurred during the subject rollover crash. Other improved ROPS structure designs were installed on the exemplar vehicle and subjected to the same 30-inch inverted drop conditions. An improved ROPS design fabricated out of higher strength steel and thicker tubing, resulting in a weight penalty of only 30lb, reduced the vertical intrusion on the impacted side of the ROPS by 81–94%. This testing and accident analysis suggests that the existing quasi-static test methods used to validate ROV rollcages is insufficient at addressing the forces and energies seen in many ROV accidents. The inverted drop test proved to be a useful method to assess ROV ROPS and reproduce real-world damage patterns. Simple fixes to the ROPS structure provided a dramatic increase in occupant survival space in the inverted drop tests conducted.

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### ROPS, ROV, Inverted Drop Testing

## D28 A Comparison of Restraint Load Marks for Retained vs. Ejected Occupants in a Fatal Rollover Crash

*David Porta, PhD\*, Bellarmine University, Louisville, KY and Corporeal Consulting, Louisville, KY*

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**Learning Overview:** After attending this presentation, attendees will better understand the relationship of vehicle investigation to the determination of injuries in a rollover crash scenario.

**Impact Statement:** This presentation will impact the forensic community by providing important information that may assist injury reconstructionists with the determination of restraint use, its relation to driver versus passenger, and ejected versus retained occupants.

On a clear fall afternoon, police were called to a rural highway in a Midwestern state to investigate the report of a male subject lying on the roadway. On scene, officers found a juvenile boy lying on his left side, conscious but unresponsive, with an obvious head injury. Approximately 30 feet up the road was a 2001 domestic sports car upside down in a shallow ditch area. A 34-year-old female was found restrained and unconscious in the driver's seat. Police determined the vehicle left the right side of the roadway, for unknown reasons, struck a culvert, went airborne for 15ft and, upon landing, rolled onto its top. Furthermore, they determined the boy was ejected through the passenger window during the half roll. No airbags deployed, and it was noted that the passenger seat belt webbing was locked in an extended position. Although an Event Data Recorder (EDR) is reportedly present in this model vehicle, no download was available. The female driver died that day, and the boy survived with serious, permanent injuries.

Subsequently, a lawsuit was initiated on behalf of the surviving boy. Six months later, in the discovery phase, an injury reconstructionist was invited to examine the vehicle and determine if the restraint system functioned properly and what role it played in the occupant injuries. Vehicle-specific reports were studied to determine the history of the car as well as United States Department of Transportation (USDOT) National Highway Traffic Safety Administration (NHTSA) reports of Complaints, Investigations, and Recalls for this make/model vehicle. In order to become educated on the particular restraint system found in this car, websites with parts listings and diagrams were examined, and the seat belt mechanism from an exemplar vehicle in a local salvage lot was removed and studied. Inspection of the subject sports car revealed significant B-pillar and roof crush with intrusion into the driver's side of the cabin space; much less damage was noted on the passenger side. Examination of the restraint systems (webbings, D-rings, latch plates, etc.) for both occupants demonstrated interesting marks: one from occupant loading and one from the method used to secure the door during vehicle transport and storage. In rollovers, as well as side impacts, load marks are more common on the latch plate than the D-ring. Marks were found on the driver's latch plate only. Furthermore, the latch plate on the locked passenger belt would not reach the inboard buckle.

In conclusion, without proper preliminary research and a thorough vehicle inspection, investigators may have assumed (based on the fixed, extended passenger belt and load marks on the D-ring) that the juvenile passenger was three-point restrained and that a seat belt system failure must have allowed his ejection. However, the results in this case demonstrate that the boy was not properly restrained and his ejection was somewhat predictable. It is atypical to have a properly restrained driver die while an unrestrained, ejected passenger survives, albeit with serious permanent injuries. But this is explained by the significant level of cabin intrusion on the driver's side.

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### Seat Belt, Vehicle Inspection, Load Marks



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## **D29 Criminal Engineering: The Ugly 50-Year History of Federal Motor Vehicle Safety Standard (FMVSS) 207 and Proposals for an Upgraded Safety Standard That Will Prevent Catastrophic Injury and Death**

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Kenneth Saccalski, PhD, Environmental Research and Safety Technologists, Newport Beach, CA; Rachel Pozzi, MS, Sandia Safety Sciences, Edgewood, NM*

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**Learning Overview:** This presentation explores the poor job that automakers and the federal government have done regarding their clear obligations to the public in automotive safety. The case histories and testing will provide a clear and accurate representation of the inadequacies and facts regarding grossly inadequate FMVSS standards, as well as how agency capture has enabled automotive defects to continue unabated for decades, despite ample proof of inexpensive alternative designs.

**Impact Statement:** This presentation will be of value to engineers, scientists, criminologists, attorneys, pathologists, police, and others involved in the forensic aspects of traffic accident investigation and reconstruction.

Widespread, life-threatening vehicle safety defects related to seats and seatbelts have been ignored for decades by automakers and the National Highway Traffic Safety Administration (NHTSA). Misinformation, deceit, and ethical lapses by automakers and safety regulators have aided and abetted this debacle that is one of the leading causes of preventable adult paralysis, fatalities, and catastrophic injuries to children in the United States. This research illustrates this is due, at least in part, to agency capture of Department of Transportation (DOT) officials.

Petitions to upgrade FMVSS 207 and to establish a rear-impact occupant protection standard have been “under review” at NHTSA since 1974. This includes multiple petitions since 1989. This ongoing dangerous vehicle safety hazard has continued to occur despite ample notice of thousands of preventable deaths and countless catastrophic injuries, and scientific research clearly proving far safer, economically feasible alternative designs. Even after the debacles of seat and belt failure shown on CBS *60 Minutes* in 1992, and on other broadcast documentaries since then, automakers continue to build vehicles with grossly inadequate seats that collapse rearward at school zone speeds, which also defeat seat belts, simply because the lax safety standard 207 and reduced requirements of FMVSS 209 allows them to do so. They also continue to ignore readily available inexpensive technology that would significantly reduce the threat of crash-induced seat and belt failures. As just one example, the Minicars Research Safety Vehicle (RSV) was developed in the 1970s to prove that fuel efficient, yet extremely safe, vehicles were readily producible by automakers. The RSV had seats that could withstand adult male loading in 50mph rear impacts by a 4,000-pound rigid moving barrier. NHTSA later intentionally destroyed the evidence of the remaining RSVs. NHTSA admitted in 1996 that FMVSS 207 is “flawed and inadequate” but has yet to change the standard 26 years later.

All these areas exemplify how agency capture of the DOT has occurred in violation of public trust. It will be seen that automakers and the government have and continue to fail to provide reasonable protection to the public in the event of automotive rear impacts and rollovers. Automakers continue to provide misleading and untrue information to the public and to NHTSA safety regulators, who in turn continue to turn a blind eye to ongoing serious safety defects that are killing and maiming children and adults every week. Hundreds of static and dynamic tests have been conducted to evaluate seats, belts, and alternative designs as well as decades of forensic cases involving catastrophic injury and deaths of adults and children have been investigated. Readily available, inexpensive technology has existed for decades to reduce or eliminate these hazards. Implementation of such recommendations to improve FMVSS 207 will be described in detail.

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### **Seat Failure, FMVSS 207, Criminal Engineering**

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## D30 Failure of Suture Materials Used for a Caesarean Section

*Sarah V. Hainsworth, PhD\*, Aston University, Birmingham, United Kingdom*

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**Learning Overview:** Following this presentation, attendees will understand the mechanical properties of suture materials, their possible failure modes, and the type of evidence that is required for determining the root cause of the failure.

**Impact Statement:** This presentation will impact the forensic science community by allowing materials scientists, engineers, and surgeons to understand how sutures fail and the techniques that can be used to investigate failures and document the necessary evidence.

Absorbable sutures are widely used for internal wounds; and one common situation in which they are used is for caesarean sections after childbirth. The materials used for the sutures are usually polymer fibers made from non-toxic monomer precursors. One of the most widely used polymers is polyglactin 910, a polysaccharide made from a copolyester of lactic acid and glycolic acid. These copolyesters are harmless products that are easily hydrolyzed by body fluids over the same time scale as wound healing and are then excreted by the body.

In a number of cases, sutures have been found to have failed post-surgery, leading to traumatic consequences of the wound opening and the intestine being exposed. Patients in this scenario pursue claims against the surgeon, the hospital, and/or the suture manufacturer. In some cases, the stitch that failed is not retained, owing to the emergency nature of the surgery needed to repair the wound. This leaves the question for the expert witness of how the stitch failed when investigation of the evidence is impossible.

New suture materials can be tested to determine the strength of the materials. This presentation reports the results of tests on three types of synthetic suture materials: Monocryl™ (dyed), Vicryl™, and PDS®. Each suture type was supplied in two gauge sizes: metric 1 and metric 0, corresponding to suture diameters of 0.4mm and 0.35mm, respectively. Monocryl™ and PDS® are monofilaments (a single strand), while Vicryl™ is a braided filament. All three types of sutures were obtained from Ethicon®, which is a division of Johnson and Johnson Medical Ltd. The suture materials were found to have very high tensile strengths between 1,200 to 1,400MPa.

The sutures tested are all known to lose their strength *in vivo*, as a consequence of the hydrolysis of the polymer as the material is absorbed into the body. The manufacturer's data were used to calculate the retained strengths after a period *in situ*.

In addition to the quasi-static tensile tests, tests at high strain rates were conducted to see whether “coughing” by the patient might lead to conditions under which the sutures would fail. The suture materials were found to be strain-rate insensitive for the testing speeds used here. The possible loading on the sutures from the soft tissue were estimated and found to be far lower than the forces required to exceed the tensile strength of the fiber.

Possible failure modes for sutures include: failure at knots or other attachment points; incorrect technique by the surgeon leading to the knot not being properly tied; the stitch not being placed correctly in the soft tissue; failure of the suture material itself from damage during the surgery or handling in the operating theatre; a quality-control issue in the polymer precursors; or an included defect during manufacture of the suture material.

In the absence of evidence retained during the medical emergency, it is not possible to attribute fault. The work highlights the need for evidence collection and failure analysis through the retention of failed products, or even good, contemporaneous photographs of failures that would allow the expert to comment more confidently about the cause of the failure.

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### Fracture, Suture, Casearean

## D31 Fatal Compression of the Torso Due to the Sudden Trapping of a Saree in the Rotator of a Pulse Mill: An Unusual Incidence During the Performance of a Harmless Activity With a Careless Attitude

Nilesh Tumram, MD\*, Government Medical College Chandrapur, Chandrapur, Maharashtra, India

**Learning Overview:** After attending this presentation, attendees will be familiar with the fatal compression of torso by long loose clothes like a saree getting trapped in a moving object like the rotators of a pulse mill, causing sudden fixation and loss of ventilatory capability.

**Impact Statement:** This presentation will impact the forensic science community by creating an understanding of the harm caused by compression of the torso by clothing trapped in a moving object. Autopsy findings such as patterned abrasion over the entire torso due to compressive force caused by the fabric of the saree and the internal structure damages can be of important forensic significance. Presented is a case study for highlighting this occurrence and its possible significance to correlate with such incidences.

In compression asphyxia, the respiration is prevented by external pressure on the body. Different cases of compression asphyxia in different circumstances have been reported, but compression asphyxia due to entrapment of a victim's saree in the rotator machine of a pulse mill is rarely reported.<sup>1-3</sup> Diagnosis is largely made by circumstantial evidence supported by traumatic injuries over the body explaining the death. In present case, a 38-year-old female victim who was assigned to cleaning activities in a pulse mill was trapped in an upright position due to sudden compression and fixation of her saree trapped in the rotator of a pulse mill; she was unable to rescue herself due to the force and power of the rotator, leading to compression of her torso and the subsequent fatality.

Autopsy findings showed patterned abrasion over the entire torso in conformity to the compression of the clothing worn by the victim, which included a thin apron, a saree, and a blouse. The internal visceral organs, such as the lungs, liver, and kidneys, showed hemorrhages. Ribs were fractured. Hemorrhages were also noted in the musculature of the torso and neck. There was tearing and transaction of coils of small intestine and also tearing and hemorrhages in the mesentery. The interesting findings were the spinal injuries at the cervical and thoracic level occurring due to the rotational movement of the rotator machine causing transmission of a spinning effect and strain over the spinal column, leading to its damage. The victim was unable to change position due to getting caught by her own clothing draped around her body and entrapped in the machine.

The history, circumstances, and findings observed in this particular case are very rare. Postmortem examination of victims of such incidences show relatively different external findings, which can be of significant forensic importance and can become crucial. In cases in which such accidental compression of the torso occurs, a detailed inspection of circumstances and bodily injuries may provide invaluable information.

### Reference(s):

1. Eren, B.; Türkmen, N.; Fedakar, R: An unusual case of thorax compression. *J Ayub Med Coll Abbottabad*. 2008, 20(1):134–135.
2. Richards, E.C.; Wallis, N.D. Asphyxiation: A review. *Trauma*. 2005, 7:37–45.
3. Shamblin, J.R.; Mcgoon, D. Acute Thoracic Compression With Traumatic Asphyxia Report of Six Cases. *Arch Surg*. 1963;87(6):967-975.

### Fatal Compression, Torso, Saree

## D32 The Biomechanics of Strangulation: Should Limits Be Placed on Restraint Tactics?

*Harold Franck, MSEE\*, Advanced Engineering Associates, St. Petersburg, FL; Darren Franck, MSME, Advanced Engineering Associates, Charleston, WV*

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**Learning Overview:** This presentation is intended to cast some clarity on the issue of this restraint system by quantifying the amount of force that may be produced in such a manner by the different methods that are employed.

**Impact Statement:** Biomechanical engineers and law enforcement regulators should benefit from this analysis of the dangers posed by these techniques.

Recent events in the news and the court systems have placed revealing evidence that choke-type holds and restraints have been widely used by law enforcement officials to subdue individuals who may have been involved or were suspected of criminal offenses. In most instances, law enforcement individuals have claimed that such measures are warranted and necessary to restrain combative subjects, even though the suspected perpetrators are handcuffed or restrained by multiple law enforcement individuals.

Historically, this technique of subjugation was used and taught in the martial arts and in law enforcement. In the martial arts, the technique is used to cause the subdued individual combatant to “give” by tapping out to notify the subduer and referee that the contest is over. At that point, the contest is over and the forces restraining the air and blood passages through the neck are released. Additionally, in martial arts contests, a referee controls the action and may end the contest at any point when a potential for serious injury arises. Unfortunately, that is not the case when law enforcement is involved in subduing a suspected perpetrator. In such cases, law enforcement is the sole judge on the amount of force and time that the restraint is applied. Fortunately, some police and law enforcement agencies have realized the dangers of this restraint system and no longer train and allow the restraint method.

This study conducted scientific tests utilizing an anthropometric test dummy to measure the forces that are applied by a variety of holds such as produced by the hands, the forearm, and the knee that can result in loss of blood flow to the brain and loss of air to the lungs. Manual strangulation is produced by using the hands, forearm, knee, or an object such as a baton that leads to the interference of blood flow to the brain and or a compression of the air passages to the lungs. When air is reduced to the lungs, the individual being strangled has the sensation of not being able to breathe and may struggle violently.

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**Asphyxia, Chokeholds, Force**

## D33 The Fatal Explosion of a Rubber Plug

*Helmut Brosz, BSc\*, Forensic Science International Group, Markham, Ontario, Canada and Institute of Forensic Electrical and Mechanical Sciences, Markham, Ontario, Canada; Renee Knight BSc, Institute of Forensic Electrical and Mechanical Sciences, Markham, Ontario, Canada*

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**Learning Overview:** This presentation will provide an understanding of how a widely used inflatable rubber plug device that is used to temporarily block/close off liquid-carrying pipes of various diameters can become a deadly projectile when it ruptures *in situ* and impacts a person. The physical, theoretical, practical, and possible criminal aspects of the issues will be presented.

**Impact Statement:** This presentation will impact the forensic science community by improving the ability of investigators and Authorities Having Jurisdiction (AHJ), including lawyers, medical examiners, and forensic engineers, to identify the investigative issues and key evidence required when investigating inflatable rubber plug fatalities and incidents.

On Friday, November 10, 2017, an employee of a plumbing company suffered a fatal injury while working to repair a corroded 24" spiral steel drain pipe. This pipe had collapsed and caused a roadway to cave in. Earlier that day, an inflatable pipe plug was installed in the drain pipe to block the flow of water from a pond to the excavation site. This enabled the employee to work on the repairing the drain pipe.

The plumbing company excavated the area around the cave-in to expose the 24" drain pipe. It was determined that the steel pipe was severely corroded; this caused water to flow from the pond to the excavation via the pipe while a pump was pumping the water out of the excavation site. The pipe was then blocked with the subject inflatable rubber plug in its protective rubber sleeve. The subject plug and another plug were inflated, with their protective sleeves, from the same regulated compressor and remained inflated for approximately two hours at a pressure not exceeding 35psi. While the employee was working in the excavation close to the pipe facing the pond, the inflated subject pipe plug suddenly exploded and was ejected by means of the compressed air within the plug.

The 35lb plug struck and killed the employee. The protective sleeve remained in the steel pipe, whereas the subject plug was found in the excavation area. Both were retrieved for forensic analysis.

Through ongoing forensic investigation, the following possibilities and issues were investigated: the inflatable plug potentially had (1) criminal tampering, (2) manufacturing defects, (3) been overinflated, (4) pre-existing damage, (5) inadequate warnings, (6) a lack of production tests and type tests, or (7) failed to follow the prescribed safety precautions. This forensic investigation and analysis of a rubber plug was established to find the most likely root cause(s).

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### Rubber, Plug, Fatality

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## D34 An Exotic Treatment for Chronic Paralysis by Kerosene Oil Ingestion: An Unusual Fatality by a Bizarre Form of Self Medication

*Nilesh Tumram, MD\*, Government Medical College, Chandrapur, Chandrapur, Maharashtra, India*

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**Learning Overview:** After attending this presentation, attendees will be able to identify the uniqueness of some exotic treatment for chronic illness like paralysis that are not only a weird form of self-medication, but also may prove fatal.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of exotic treatments that are peculiar forms of self-medication and may be of forensic significance if such cases were found in places without no witness to incident. Presented is a study for highlighting such an occurrence that has rarely been mentioned in available literature.

Usually most of the literature worldwide mentions kerosene poisoning due to accidental exposure or consumption. Sometimes it is consumed for attempted suicide. However, kerosene consumption for medicinal purposes is very infrequently reported and has not been seen in the available literature. A 10-year study was conducted to ascertain the practice of consumption of kerosene by individuals for medicinal purposes. It was found that almost in all cases, the person was suffering from one or other form of paralysis. Due to the chronic nature of the illness, the patient or their relatives became frustrated to the extent that they tried every possible measure to get cured of the illness. In the process, they not only try the traditional ayurveda or homeopathy treatment, but also some exotic treatments suggested by medical quacks.

Their treatment can be as odd as the consumption of cow's urine or cow-dung to some poisonous plants such as the milk of calotropins or the fruit or leaf juice of oleander plants, etc.<sup>1</sup> In continuation to similar practice, cases of fatalities by kerosene oil allegedly given or self-consumed for treatment of such chronic illness have been noticed. The dose of kerosene used was as small as a one teaspoonful (5ml) to as large as a 200ml bottle. In all these cases, the deceased were medicolegally autopsied. The lungs and respiratory tracts were the major organs to exhibit changes such as marked congestion and edema, while the heart and other organs showed multiple petechial hemorrhages.

This presentation will also discuss the importance of possessing the knowledge of such manners and causations of injuries leading to the death of victims and their relevance when dealing with these medicolegal cases.

**Reference(s):**

1. Afroze, C.A.; Rahman, T.; Jahan, R.; Rahmatullah, M. Evaluation of a folk medicinal cure for stroke induced paralysis and pain. *Journal of Medical Plants Studies*. 2018, Vol. 6, Issue 2.

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### Exotic Treatment, Kerosene Oil, Chronic Paralysis

## D35 The Determination of Lumbar Disc Injury Potential from a Chair Collapse Incident

*Michelle Hoffman, MS\*, Forensic Injury Analysis, LLC, Tempe, AZ; Robert Anderson, MS, Biomechanics Analysis, Tempe, AZ*

**Learning Overview:** After attending this presentation, attendees will better understand the importance of both the magnitude and direction of the loading on the lumbar spine with respect to disc injury potential resulting from a chair collapse incident.

**Impact Statement:** The presentation will add data collected from human volunteer testing for utilization of the forensic science community.

A 25-year-old, 5'7", 150-pound woman in a furniture store sat in a foldable lawn chair on display while holding her 1.5-year-old child. The chair's front dowel had been removed, so when she sat, the fabric pulled through the frame, allowing her to fall to the floor. Her fiancé took a photo after the incident before he helped her up (Figure 1). She first sought medical care six days later. The following question had to be answered: Would an L5-S1 disc herniation result from the loading experienced during the described chair collapse? This presentation describes how this question was addressed scientifically.



Figure 1 - Photo by fiancé.

The woman testified she held her child in front and sat “as you would in a normal chair,” with the seat bottom’s leading edge right above her calf. Afterward, she couldn’t get herself out, “because of the angle it was at.” She testified she first felt pain 1.5 hours later.

Research has shown that the only mechanism that produces lumbar disc injury from a single traumatic event is combined compression (z-axis or axial loading) and hyperflexion beyond the physiological range of motion.<sup>1</sup>

Testing was conducted to measure the ground reaction forces and pelvic/lumbar accelerations experienced by a 49-year-old, 5' 7", 150-pound female surrogate, who sat in an exemplar chair with the front support dowel removed. She held a 20-pound rice sack to represent the child. Accelerations were measured in the x (fore-aft), y (left-right), and z (up-down or axial) directions. Four tests were also conducted on a 50-year-old, 5'7½", 200-pound male surrogate with no rice bag. Two flooring conditions were used: (1) rug on artificial turf on level concrete; and (2) rug on artificial turf on plastic decking on level concrete. For each condition, two tests were performed: one with a load cell between the concrete and the next layer of flooring, and one without a load cell. Pelvic/lumbar accelerations were measured in all tests. All tests were recoded with high-speed (500fps) video.

A photograph of the female surrogate after falling through the chair is shown next to the post-incident photo in Figure 2.

These photos, as well as a side view of the surrogate (Figure 3), made it clear that they did not land on their ischial tuberosities (sitting bones), but rather, their sacrum/low back areas made initial contact.



Figure 2- Post-incident photo (left) and surrogate photo (right) after chair collapse.



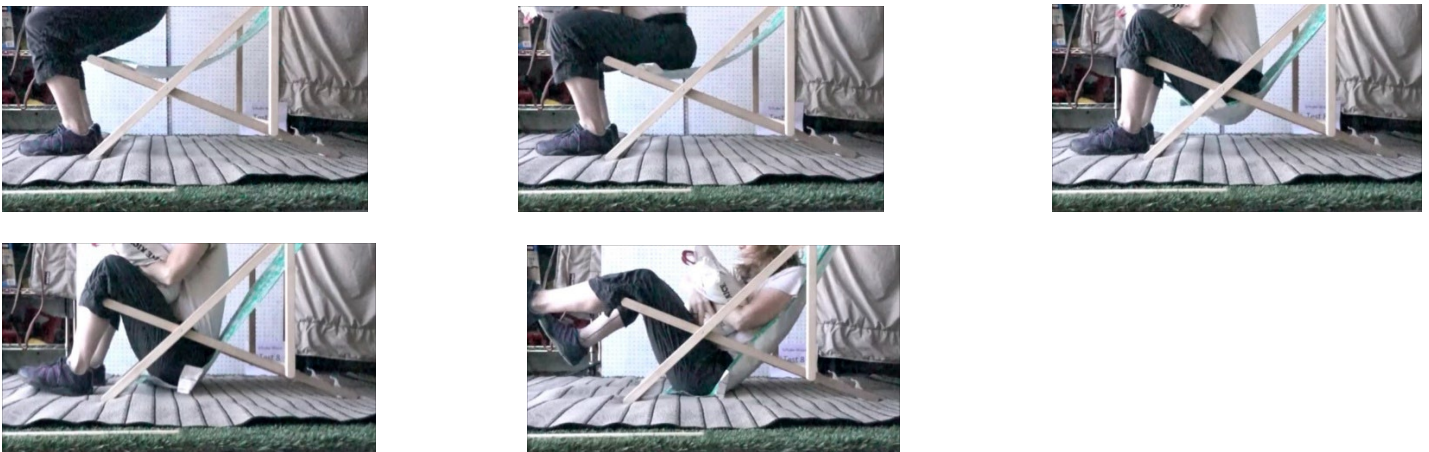
Figure 3 - Side view of surrogate showing ground contact with sacrum/low back.

Test	Subject	Surface	Load Cell	Peak Pelvic Accel (g's)				FRF (lb)	Notes
				X	Y	Z	Res		
1	A	C,D,T,R	N	-7.8	-6.3	-1.0	9.4	N/A	
2	A	C,T,R	N	-13.7	-9.7	-0.7	15.3	N/A	
3	A	C,D,T,R	Y	-7.3	-5.0	-1.3	8.0	779	
4	A	C,T,R	Y	-7.1	-5.0	-1.5	9.0	804	
5	B	C,T,R	Y	-2.7	-3.6	-1.9	4.6	446	
6	B	C,D,T,R	Y	-1.0	0.7	-0.5	1.4	0	forgot to remove dowel
6B	B	C,D,T,R	Y	-3.2	-3.3	-0.4	4.0	308	
7	B	C,T,R	N	-3.4	-2.6	-1.1	4.2	N/A	
8	B	C,D,T,R	N	-2.8	-1.5	-2.2	3.9	N/A	

A = 50-year-old, 5'7.5", 200-lb male  
 B = 49-year-old, 5'7", 150-lb female  
 C = Concrete  
 D = Plastic decking  
 T = Artificial Turf  
 R = Rug  
 FRF = Floor Reaction Force  
 Res = Resultant Acceleration

Axial loading along the spine was minimal, and the largest accelerations were in the fore-aft direction for the pelvis/lumbar spine. Lumbar z-accelerations ranged from 0.4–2.2gs, while x-accelerations ranged from 2.7–13.7gs. High-speed video revealed that the shins were initially supported by the chair's front crossbar, and the pelvis rotated rearward and translated forward prior to, during, and after contact with the floor, which was consistent with the acceleration test results. Video stills from Test 8, which had the highest vertical acceleration, are shown in Figure 5.

Figure 4 - Chair Collapse Test Results.



5. Still images from high-speed video during Test 8.

In conclusion, analysis of the information available and testing of human surrogates with an exemplar lawn chair revealed that the magnitude and direction of the force applied to the pelvis/low back from the subject incident was not causal in an L5-S1 disc herniation.

**Reference(s):**

<sup>1</sup>. Adams, M.A.; Hutton, W.C. Prolapsed Intervertebral Disc: A Hyperflexion Injury. Spine, Volume 7, Number 3, pp. 184-191, 1982.

**Lumbar Disc Injury, Chair Collapse, Human Testing**



## D36 Technicalities of the Shotgun, Its Ammunition, and the Forensic Implications

*John Nixon, MBA\*, ARC, Bippus, IN*

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**Learning Overview:** This presentation is aimed at the non-specialist/semi-technical attendee and particularly jurists involved in criminal and civil litigation involving shotguns. Attendees will gain an appreciation of the basic science and technology behind shotguns and shotgun ammunition. The fundamental differences between shotguns and other classes of firearm will be explained in terms of, among other things, ease of use, accuracy, wounding potential, versatility, and terminal performance. Media-perpetrated myths regarding shotguns and their performance will be dispelled.

**Impact Statement:** City-dwelling jury members are likely to have very little knowledge of shotguns, other than the misinformation they have gleaned from TV shows and movies. The justice system will benefit from having courtroom practitioners who have a realistic basic understanding of shotguns and their ammunition and who are able to convey that knowledge to juries so they can dismiss the myths and make determinations based upon reliable knowledge and facts.

Rifles and pistols are single-projectile weapons that launch a single projectile that is spin-stabilized and retains significant velocity and energy over extended ranges. It is difficult to hit small moving targets with these single-projectile weapons, and they can inflict serious or fatal injuries at distances way beyond most intended targets.

Shotguns and their ammunition are radically different to other classes of firearm. Shotguns have been around for hundreds of years. They enable a shooter to more easily hit a moving target due to the multiple pellets they launch toward the target that form a cloud of small projectiles. The rapid deceleration and resulting short range of the pellets ensures that they have a relatively short danger zone, and this makes them suitable for use in semi-urban environments. Historically, shotguns have been extremely popular for vermin control and bird hunting.

In the early years, shotguns were single or double-barrel, but by the end of the 19<sup>th</sup> century, pump action and semiautomatic shotguns had been developed, and by WWI, they had been adopted by military units for use in urban and trench warfare.

Shotguns were widely adopted by police departments due to their devastating close-range effects and relative safety at medium ranges. Tens of millions of shotguns are currently in circulation in the United States, and while many are utilized for vermin control/crop protection, a large number are purchased for personal protection and security. Inevitably, some are used in crimes, and there are accidents.

Shotguns are considered to be very versatile weapons, because one gun can be used with many different types of ammunition. Small birdshot ammunition can be used to control vermin, while the same gun can be used with buckshot for security duty, and with a single projectile “slug” for deer hunting or protection from large predators.

The shotgun has garnered quite a reputation due to the dramatic misrepresentations of Hollywood and barroom talk. It is almost routine to see TV shows and movies where people shot with a shotgun fly backward through the air, cars explode upon impact, and holes the size of wagon wheels are blown through doors and walls.

From a forensic perspective, the questions most asked relate to safety (design and/or manufacture defects), range of fire determination, and shot size and weight used. The range of calibers (gauges) and the sheer diversity of ammunition available can be quite a challenge. This presentation utilizes technical data and presents examples from actual case studies to illustrate the principles involved.

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### Shotgun, Shotgun Ammunition, Shotgun Myths

## D37 A Visualization of Organic Gunshot Residues with Hyperspectral Imaging (HSI) and Correlation Analysis

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**Learning Overview:** After attending this presentation, attendees will understand how hyperspectral imaging can be used to visualize organic gunshot residues and that the method has the potential to be widely useful in forensic science.

**Impact Statement:** This presentation will impact the forensic community by providing a method that can visualize organic gunshot residues and indicate the possibility of firearm use even when no bullets are found at a crime scene.

“Gunshot residues” is a general term for particles produced by shooting and generally refers to metallic particles derived from bullets and priming powder. However, in a broad sense, it also includes organic materials such as unburned and partially burned powder. In this study, hyperspectral imaging and correlation analysis were tried to visualize partially burned powder as organic gunshot residues.

Hyperspectral imaging is widely utilized in the forensic science field because it allows non-contact measurement without destroying the sample. As applications, detection of latent fingerprints, separation of overlapped fingerprints, visualization of blunt trauma, and bloodstains have been reported.<sup>1,2</sup> The data measured by hyperspectral imaging are layered images with high-resolution wavelength information, called a hypercube. By using the differences in fluorescence spectra resulting from excitation light irradiation, it is possible to visualize and discriminate samples.

A Automatic Colt® Pistol (45ACP) with a Lead Round Nose (LRN) was fired at a white cotton towel from a shooting distance of 5cm to produce a sample in which partially burned powder was dispersed around the projectile entrance. The measurement was performed by HSI using 415nm and 505nm Light-Emitting Diodes (LEDs) as excitation sources. The long-pass filter used was 450nm for 415nm excitation and 560nm for 505nm excitation. The scanning time of HSI is 190sec The effective scanning area is 3cm x 3cm.

It was difficult to identify the dispersion of powder with the naked eye since the area around the projectile entrance was discolored black due to the adhesion of lead and other substances. In the case of 505nm excitation, the position of partially burned powder was well visualized because of the remarkable difference in fluorescence between the background and the powder. On the other hand, in the case of 415nm excitation, the prominent background fluorescence interfered with the identification of the powder. However, by normalizing the hypercube in the wavelength direction, good visualization of the powder was achieved.

The originality of this study is that the dimension reduction by principal component analysis was applied to the correlation matrix between fluorescence spectra of visualized particles. This method will enable clustering that takes into account the characteristics of particles and will provide more useful information to the crime scene.

### Reference(s):

1. Nakamura, A.; Okuda, H.; Nagaoka, T.; Akiba, N.; Kurosawa, K.; Kuroki, K.; Ichikawa, F.; Torao, A.; Sota, T. Portable hyperspectral imager with continuous wave green laser for identification and detection of untreated latent fingerprints on walls. *Forensic Sci. Int.*, 254, 100-105, 2015.
2. Schuler, R.L.; Kish, P.E.; Plese, C.A. Preliminary observations on the ability of hyperspectral imaging to provide detection and visualization of bloodstain patterns on black fabrics. *J. Forensic Sci.*, 57, 1562-1569, 2012.

### Gunshot Residues, Hyperspectral Imaging, Correlation Matrix

## D38 An Accessible Open-Source Implementation of the Congruent Matching Cells Algorithms for Cartridge Case Analysis

Joseph Zemmels, MSc\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Department of Statistics, Iowa State, Ames, IA; Susan VanderPlas, PhD, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE; Heike Hofmann, PhD, Center for Statistics and Applications in Forensic Evidence, Ames, IA and Department of Statistics, Iowa State, Ames, IA

**Learning Overview:** Attendees will learn about the open-source implementation of the Congruent Matching Cells (CMC) algorithm in the R package *cmcR*. The algorithm is implemented in a modular form; exposing relevant parameters. Attendees will learn about methods to evaluate different parameters. Additionally, attendees familiar with the R statistical programming language will learn how to use the CMC method by way of the *cmcR* package.

**Impact Statement:** Computational pattern-matching algorithms are becoming more important in many forensic science disciplines. It is important that such algorithms are auditable by the legal and forensic science communities. The open-source *cmcR* package is written to be transparent, auditable, and user-friendly. The process by which the CMC methods were implemented in the *cmcR* package can be used as a template for future open-source implementations of pattern-matching algorithms. Additionally, changes to the original CMC method are developed that result in an improved classification rate.

Scientific research is driven by the ability to use methods, procedures, and materials from previous studies and further this research by adding to it. As the need for computationally intensive methods to analyze large amount of data grows, the criteria needed to achieve reproducibility, specifically computational reproducibility, have become more sophisticated. In general, prosaic descriptions of algorithms are not detailed or precise enough to ensure complete reproducibility of a method. Results may be sensitive to conditions not commonly specified in written-word descriptions, such as implicit parameter settings or the programming language used. To achieve true computational reproducibility, it is necessary to provide all intermediate data and code used to produce published results.

In this presentation, a class of algorithms developed at the National Institute of Standards and Technology (NIST) to perform firearm evidence identification on cartridge case evidence known as the CMC method and its variants is considered.<sup>1-3</sup> To date, only textual descriptions of these algorithms have been published. The first open-source implementation of the CMC methods in the R package *cmcR* is introduced. The *cmcR* package is structured as a set of sequential, modularized functions intended to ease the process of parameter experimentation. The *cmcR* package and a novel variance ratio statistic are used to explore the CMC methodology and illustrate problems that arise when computationally ambiguous algorithm descriptions are provided. A training/test set validation procedure is introduced and used for an evaluation of parameter settings. Additionally, *cmcR* is used as a foundation to develop changes to the CMC methodology. The updated algorithm results in an improved classification rate compared to the original CMC methods.

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### Firearm Evidence, 3D Topographic Measurements, Pattern Matching

## **D39 A Multifaceted Method for Evaluating the Driving Ability of Elderly Drivers: Toward Identifying Causes of Accidents Involving Elderly Drivers and Broadening the Recognition of Negligence Types**

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**Learning Overview:** After attending this presentation, attendees will be able to understand the relationship between the results of simple tests and personal attributes and the driving ability of elderly drivers, which will help improve negligence recognition in traffic accident reconstruction.

**Impact Statement:** This presentation will bring a new convergence of disciplines to the forensic science community by demonstrating the usefulness of multidimensional driving ability assessment of elderly drivers in traffic accident investigation and reconstruction.

In the current situation, the effects of various ability declines and diseases along with aging have not been sufficiently considered in determining the negligence of drivers in traffic accident reconstruction. In the first place, the technology for evaluating driving ability from various abilities and tendencies such as physical characteristics, which includes abilities such as brain functions, and social characteristics, which includes lifestyle habits, has not been established.

For example, in Japan, cognitive function tests have been conducted since 2007 for drivers aged 75 or older when they renew their licenses; however, the number of accidents involving elderly drivers has not decreased at all. In future, the introduction of a practical driving test at the time of license renewal for the elderly is expected to stop drivers with poor driving skills; however, even so, it is not sufficient to evaluate driving abilities, which involves a wide range of abilities such as brain processing capacity and social characteristics.

Therefore, since 2008, the Fujikawaguchiko Town Senior Driver Support Project has been continually investigating the relationship between physical functions, brain functions, and lifestyle habits of the elderly and their driving abilities and examining the changes over the years. As a result, it has been clarified that there is a relationship between the results of risk avoidance tests using a simplified Driving Simulator (DS) and the results of simple tests such as the Trail Making Test (TMT), which measures visual search processing ability, and the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC), which evaluates the life functions of the elderly, as well as personal attributes obtained from questionnaires about driving. Among them, a significant relationship between the DS score obtained from the simplified DS and the time required for TMT Part-B was obtained ( $p < 0.005$ ), and all participants whose TMIG-IC score was less than 11 points showed a sharp decrease in DS score.

This study proposes indicators that can quantitatively evaluate the driving ability of elderly drivers from various perspectives by conducting longitudinal and cross-sectional analyses of the relationship between the results of simple tests such as TMT and TMIG-IC and the results of risk avoidance ability tests. In the near future, indices will be proposed that can be used to quantitatively evaluate the driving ability of elderly drivers from various perspectives by analyzing the relationship between the results of these indices and the results of risk-avoidance tests. In addition, by analyzing these indices and data on accidents involving people with a history of accidents, violations, or pre-existing conditions such as epilepsy, data will be provided that will be useful in determining the negligence of the perpetrators of traffic accidents. Furthermore, by using these results as a reference, it is considered that simple tests similar to TMT and TMIG-IC can be administered to the perpetrators of traffic accidents at the elderly person's training course at the time of license renewal and to the perpetrators after the accident to evaluate their respective abilities and to assess the human factors of the accident in detail.

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### **Traffic Accident Reconstruction for Elderly Driver, Trail Making Test, Activity Capacity Index**

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## **D40 A Study of Injury Risk Assessment Methods for Pediatric Impacts with the Floor: An Estimation of Acceleration and Load the Human Body Is Subjected to Using Multi-Body Dynamics**

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**Learning Overview:** After attending this presentation, attendees will have learned about injury risk evaluation methods using multi-body dynamics analysis.

**Impact Statement:** This presentation will impact the forensic medicine and forensic engineering community by providing a method for injury risk evaluation using multi-body dynamics analysis.

In Japan, the number of cases of physical abuse of children has been increasing every year. Currently, physical abuse is judged based on the testimony of the victim and the diagnosis of the physician. Since this method of determining physical abuse is qualitative, there is a need for an objective and quantitative evaluation of the external force applied.

In previous studies, methods that estimate the external force from the degree of damage have been reported. However, it has not been possible to determine what kind of behavior caused the external force. Therefore, it is considered that a method that estimates the situation at the time of injury is required. By using previous research and multi-body dynamics analysis, it is considered possible to estimate the situation at the time of injury based on the degree of injury, and quantitatively determine the physical abuse of young children. This study proposes a method to estimate the risk of injury by simulating a child falling using multi-body dynamics analysis with MAThematical DYnamic MOdels (MADYMO) and calculating Head Injury Criteria (HIC) and other parameters.

In order to perform the analysis with MADYMO, it is necessary to determine the contact characteristics between the child dummy model and the floor (flooring, concrete, etc.). Therefore, this study adjusted the analysis parameters so that the acceleration waveform would be the same in the same analysis as in the experiment using the head impactor. In the experiment, the head impactor was dropped freely from a height of 90cm and collided with each floor. In the analysis, a sphere with the same radius and mass as the head impactor was dropped freely and collided with each floor under the same conditions as in the experiment. As a result, this study was able to determine the contact characteristics between the child dummy model and each floor.

In order to verify the accuracy of the MADYMO analysis, this study also performed the same analysis as in the experiment using a 3-year-old dummy doll (Hybrid III3YO) and compared the results. In the experiment, a Hybrid III3YO was dropped freely from a height of 90cm in a prone position and collided with the floor (flooring, concrete, etc.). In the analysis, MADYMO was used under the same conditions as in the experiment. As a result, this study was able to measure the same level of head acceleration in the experiment and analysis. Therefore, it was confirmed that the accuracy of the analysis was relatively good when the head first collided with the floor. The results of this study can be used to estimate the situation of child abuse from the degree of injury with higher accuracy.

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### **Injury Risk, Estimating Children's Behavior Through Simulation, Child Abuse**

## D41 The Forensic Approach in the Analysis of Trauma: The Application of 3D Technology in the Reconstruction of Death

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**Learning Overview:** After attending this presentation, attendees will understand the role of 3D technology in cases of trauma.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of 3D motion capture with circumstantial data in the evaluation of dynamics of traumatic death.

The effects of trauma are classified into general and local, direct and indirect, immediate and consecutive, primary and secondary, and anatomical and functional. The severity of the trauma depends on the mechanical force acting on the anatomical region and the type of anatomical region affected. There are many traumas in forensic pathology, from cutting or slashing, firearms, blunt instruments, explosions, crushing, precipitation, road accidents, and electrical or thermal causes. The autopsy always allows us to clarify the causes of death in these cases and to analyze in detail the characteristics of the injuries produced by trauma. However, it is increasingly difficult to analyze the ways in which the traumatic event occurred with respect to the surrounding environment and with respect to some variables that are part of the dynamic. In these cases, the role of the forensic pathologist becomes crucial to reconstruct the incident. Solving a crime always requires a systematic methodology. It proves indispensable especially when the dynamics of crime are complex; for example, when the weapons of the crime are multiple, there are no traces of the killer, the injuries on the victim are numerous, the testimonies of the suspects are doubtful or improbable. In these cases, investigators may have difficulty in reconstructing the event with certainty.

In recent years, forensic science has experienced a rapid development in the application of new computer technologies for crime scene reconstruction. In 3D technology, Motion Capture (MOCAP) enters the forensic field experimentally. It consists of a 3D virtual model based on motion capture. MOCAP is the recording of the movement of the human body (or other movements) that allows for virtual playback. This study reports a series of cases of traumatic deaths where it was necessary to clarify the circumstances and the dynamics of the events. In all cases, the reconstruction of the trauma was applied through 3D technology with the application of 3D MOCAP. This study proposes a new virtual method useful for reconstructing the dynamics of complex cases.<sup>1-4</sup> The proposed model is not routinely used in forensic medicine, and through the analysis of these cases, it demonstrates its applicative utility. In particular, the forensic purpose is to compare the lesions analyzed during the autopsy or the victim's visit with the possible dynamics compatible with the environments in which the event occurred, with the testimonies and the types of weapons used.

The experimental 3D model shown has several other advantages: the ability to perfectly keep a copy of the crime scene and its environment; the ability to easily compare various murder dynamics; being able to combine the reconstructed dynamics with the testimony; and being able to use video as judicial evidence in court. This study demonstrates that the 3D method must be able to be used in the forensic field; the crime scene is not a film and not a fiction; the reconstruction must always refer to the scientific evidence found on the scene or on the victim and cannot under any circumstances build from scratch a dynamic that cannot be demonstrated on the scene with scientific evidence. Therefore, the method represents a tool and a key to reading a piece of evidence found in forensic investigations. Doubt is the circumstance for which the forensic pathologist has reason to exist, but at the same time, it is the event that the forensic pathologist must cancel through the application of scientific rigor of the method, with the help of technology.

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### Forensic Sciences, 3D Technology, Trauma

## D42 Macro Photography in Forensic Pathology: A Useful Tool to Focus on Skin Lesions

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**Learning Overview:** After attending this presentation, attendees will better understand the value of applying macro photography in the field of forensic pathology.

**Impact Statement:** This presentation will impact the forensic science community through the presentation of a series of macro images relating to skin lesions, with particular reference to the acquisition methodology and the benefits that can be obtained in terms of evidence in the course of forensic pathological investigations.

Macro photography is a branch of traditional photography, generally adopted in the representation of insects or small objects. From a technical point of view, a photograph is defined as a macro when the photographed object is represented in its original size or larger on the sensor of the camera body. It is possible thanks to the use of particular photographic lenses able to focus on objects very close to the observer and, compatibly with the limits of the used instrumentation, allows a considerable magnification capacity and the collection of a large amount of details.

This report presents a case series of autopsies in which macro photography was used in the determination of fine details of cutaneous lesions. The photographs were captured using a camera body with Advanced Photo System type-C (APS-C) format (23.5mm x 15.6mm) Complementary Metal Oxide Semiconductor (CMOS) sensor, a 105mm F/2.8 1:1 macro lens and a macro led ring flash. All images were captured using manual settings and focus; the acquisition settings used were significantly affected by many external factors, variable for each individual lesion such as, for example, the different depths of field required, the lighting conditions, as well as the possibility of using a tripod. The survey sample includes cases of external cadaveric inspection or judicial autopsy performed at the Department of Forensic Pathology of Sapienza University of Rome. In particular, the skin lesions studied, belonging to at least 20 cases of judicial interest, include cases of sharp and blunt injuries, gunshot wounds, and ligature marks.

The analysis of the obtained images made it possible to appreciate some distinctive elements of the skin lesions difficult to observe with the naked eye, able to direct the diagnostic judgment regarding the vitality of the lesion and the productive mechanisms, such as describing the asphyxiogenic medium features in cases of violent asphyxia, reconstructing the direction and characteristics of the blade in the case of sharp injuries, and identifying the typical characters of entrance and exit gunshot wounds, as well as accessory findings.

In conclusion, macro photography can be an important and practical tool in the study of skin lesions by virtue of the speed and ease of use, the low cost of the equipment, and the ease of interpretation of the observed findings. Certainly not being able to provide results comparable to the microscopic study of lesions, macro photography can be considered a preliminary study phase to it, already allowing, during the crime scene investigation or the first examination of a corpse, documentation of important semeiological findings and freezing elements for evidential purpose, with the ability to non-invasively and more easily locate small findings in the context of the entire lesion.

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### Macro Photography, Skin Lesions, Diagnostics

## D43 The Challenges in Reproducible Capture and Analysis of Cannabinoids with an Impaction Filter Device

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**Learning Overview:** Attendees to this presentation will learn about devices for the collection of exhaled breath aerosols and challenges specific to the quantitative analysis of cannabinoids.

**Impact Statement:** This presentation will impact the forensic science community by discussing how human factors and device design impact the reproducible quantitation of cannabinoids in the exhaled breath of cannabis users, which is required for reliable, unbiased chemical determination of cannabis use.

Cannabis induces decreases in executive function and motor control that make drivers more likely to be involved in crashes than unimpaired drivers.<sup>1</sup> The premise behind a “cannabis breathalyzer” is that cannabis use can be quantified with field-deployable devices akin to those used for breath-alcohol analysis; however, ethanol (EtOH) and delta-9-tetrahydrocannabinol ( $\Delta^9$ THC) have dramatically different thermophysical properties that create challenges for breathalyzer development. EtOH is soluble in blood and, compared to other drugs, a large quantity can be consumed—a standard drink contains 14g. EtOH remains in the vapor phase (i.e., does not condense) during exhalation and quantitation and blood/breath ratios have been measured in thousands of individuals. By contrast,  $\Delta^9$ THC is expected to be carried by aerosol particles based on its low volatility ( $\Delta^9$ THC is 100 million times less volatile than EtOH).<sup>2</sup> Despite its low blood solubility and volatility,  $\Delta^9$ THC has been successfully recovered by concentrating analytes from a large volume of breath, with identification and quantitation by liquid chromatography and tandem mass spectrometry.<sup>3-7</sup>  $\Delta^9$ THC spikes following cannabis use, then decreases, and has been recovered for time periods ranging from 1h to 3h following use.

Exhaled breath aerosols can be collected with surgical masks, electret filters, impaction filters, and condensate devices, which condense water vapor and volatile compounds in addition to capturing particles. The materials, dimensions, mode of action, and extraction methods influence which compounds are recovered and new designs are frequently reported, reflecting the early stage of aerosol collection. Future adoption of aerosol collection devices for evidentiary purposes requires an understanding of how human factors, ambient conditions, and device design impact cannabinoid recovery. For example, individuals differ in their production of aerosol particles, which increases with the ventilation ratio ( $V_R$ )—this is the tidal volume ( $V_T$ , which is the exhaled volume) divided by the vital capacity ( $V_C$ , which is the total volume that can be exhaled).<sup>8</sup> Standardization is limited: breath has been collected for a specific time, number of breaths, or volume.<sup>3-7</sup>

This presentation will focus on the collection of exhaled breath aerosols with an impaction filter device consisting of a wide mouthpiece followed by three parallel collectors. The cylindrical collectors are 22mm long, have a 10mm internal diameter, and contain eight baffles perpendicular to the main direction of the flow that dictate the velocity profile. The device can be used alone or connected to a spirometer. Pre-consumption and post-consumption samples were collected from experienced cannabis users consuming a single legal market product in a naturalistic, observational design; compliance with protocol was verified with blood cannabinoid measurements.<sup>9</sup> Each exhalation through the device followed a low lung volume breath hold to increase aerosol particle production.<sup>10</sup> Analytical results will include a discussion of challenges related to  $\Delta^9$ THC extraction and quantitation. The presentation will also include laboratory studies of oral fluid contamination, measured via detection of alpha-amylase activity. Numerical simulation methods, specifically computational fluid particle dynamics, will be described to provide insight into the velocity profiles and particle capture efficiency of individual collectors. Inherent human variability can ultimately be mitigated by appropriate device design and standardized breath collection and analysis protocols.

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### Breath Aerosols, Cannabis, Impaction Filters

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## D44 A Forensic Analysis of Cosmetic Smudges Using Mass Spectrometry: The Effect of Sampling, Surface, and Time

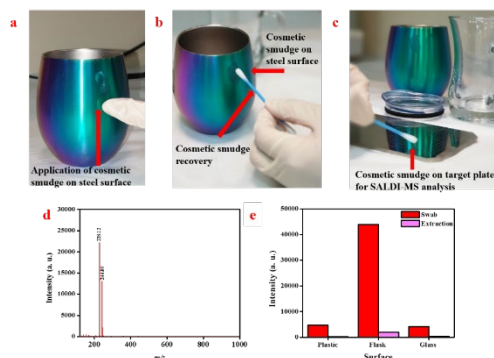
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**Learning Overview:** After attending this presentation, attendees will have learned about the results obtained on the recovery and analysis of cosmetic product smudges found on different surfaces common in every household and that can also be found in a crime scene. Cosmetic smudges have recently gained some attention and most of the focus is on the analysis of makeup products to discriminate between them using primarily spectroscopic techniques. Recently, the persistence of moisturizer products on human skin in relation to sexual assault investigations was covered using Gas Chromatography/Mass Spectrometry (GC/MS). This study broadened the research aspect by looking at different surfaces (glass, steel, and plastic) using five different cosmetic products that can be purchased without prescription and used by both genders without age restriction. In addition, to perform the analysis in an efficient way, Surface-Assisted Laser Desorption/Ionization (SALDI)-MS was employed without sample preparation and cleanup with a minute amount of sample. The results obtained are very promising and can be further used to include other products and to study their stability under different temperature and humidity conditions. Therefore, forensic analysis of cosmetic smudges can be performed directly without sample cleanup employing a small amount, which is highly beneficial for forensic applications.

**Impact Statement:** Recently, research into forensic smudge analysis has gained a lot of attention. This is motivated by the reality of crime scenes where there is no control over the quality and quantity of the physical evidence. Several researchers have focused on smudges of biological fluids and their importance in DNA analysis to convict or exonerate a suspect. Limited attention has been dedicated to the analysis of non-biological smudges recovered in a crime scene, in particular, analysis of cosmetic products that are highly relevant in crimes involving females. Therefore, for the first time, this study explored the analysis of cosmetic products using SALDI-MS using small amount of these products. The obtained mass spectra, the effect of surface on which they were deposited, and aging of the smudges were carefully studied. The smudge recovery approach in combination with SALDI-MS provides overall gains in discrimination power, analysis time, and reduces matrix effect. Moreover, the imposed lockdown during the COVID-19 global pandemic has increased the necessity for more advanced techniques that can coincide with the massive delays and sample backlog in forensic labs.

Cosmetic evidence recovered from crime scenes is emerging as a significant finding that can assist in linking or exonerating a suspect to certain crime or victim. Due to their texture and composition, cosmetics tend to strongly adhere to surfaces and persist for a long time. In this work, five cosmetic products containing different Active Pharmaceutical Ingredients (APIs) were investigated utilizing SALDI-MS. Bepanthen® cream, Reparil® gel, Eucerin® cream, Soskin® serum, and Clofen® cremagel containing dexpanthenol, diethylamine salicylate, urea, Salicylic Acid (SA), and Diclofenac Sodium (DCS) as APIs, respectively, were employed in this study. Initially, based on the signal intensity obtained for each API, the optimum extraction solvent was investigated using solvents of different polarity indices. Consequently, ethanol was recognized as the best solvent for salicylate, urea, and DCS, whereas propanol for SA, and lastly Dichloromethane (DCM) for dexpanthenol.

Often in a crime scene traces of cosmetics are encountered, therefore, Limits Of Detection (LOD) is of paramount importance. In this context, several dilutions were carried out and LOD of 300ng/mL for dexpanthenol, 30ng/mL for salicylate, 6ng/mL for urea, 1.20ng/mL for SA, and 600ng/mL for DCS were obtained. Furthermore, the Relative Standard Deviation (RSD) for each API was also calculated and the values ranged between 0.40 to 17.95%, which indicated good reproducibility. As a proof-of-concept, smudges of five cosmetics were freshly deposited on the SALDI-MS target plate in a small amount without extraction and the APIs were successfully detected. Subsequently, simulation of forensic samples by locating cosmetics smudges on three different surfaces of household items, namely plastic, glass, and stainless-steel (flask, Figure 1a), and recovered via two approaches. In the first approach, the smudges were swabbed with wet cotton (using the optimum solvent) and subjected to extraction; on the other hand, the second approach was based on swabbing the smudges with wet cotton (using the optimum solvent, Figure 1b) and placing it directly on the target plate (Figure 1c). Although in both approaches the APIs were detected, the second approach was superior and can be directly implemented in the crime scene due to its simplicity. Additionally, for more precise data interpretations, substrate control analysis was performed for the three surfaces and their contribution to the spectra was accounted for (Figures 1d and e). Finally, based on the results obtained for each surface, aging of the smudges of each product were studied over three weeks. Notably, four APIs—dexpanthenol, salicylate, urea, and SA—were detected with relatively high signal for up to three weeks, while DCS signal decreased after only one day; however, it remained detectable up to three weeks. Therefore, the results confirmed the stability of cosmetic products on surfaces for long-term periods and the superiority of SALDI-MS to detect the ingredients of such products with minimum sample handling and pretreatment.



**Figure 1.** (a) Cream smudge placed on the flask (steel surface), (b) after 30 minutes of deposition, the smudge is collected via moistened cotton swab with the optimum solvent, (c) the swab was directly placed on the target plate and allowed to dry at room temperature prior to the analysis, (d) the obtained mass spectrum of Bepanthen® cream (as an example) showing sodiated and potassiated adducts of dexpanthenol, (e) signal intensity obtained using the swab and extraction approaches on three different surfaces: plastic, steel (flask) and glass.

### Forensic Smudge Analysis, Cosmetic Products, Mass Spectrometry

## D45 Fluorescence Lifetime Imaging (FLIM) of Latent Fingerprints on Paper Emitting Strong Fluorescence at Excitation by 532 Nanometers (Nm) Pulsed Laser

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of visualizing latent fingerprints non-destructively by FLIM.

**Impact Statement:** This presentation will impact the forensic community by reporting a successful case of using FLIM for visualizing latent fingerprints on a background substrate that emits such strong fluorescence at laser irradiation that even the conventional time-resolved spectroscopy does not work.

Fingerprints are important evidence for human identification in criminal investigations. Visualization of latent fingerprints by photoexcitation using laser and then photographing fluorescence of fingerprints is a powerful method as a non-contact and non-destructive detection method.<sup>1</sup> Since background substrates of fingerprints often emit fluorescence, it becomes difficult to visualize latent fingerprints when background fluorescence is strong. Although differences in wavelength range and lifetime of fluorescence are utilized to reduce the effect of background fluorescence, it is hard to visualize latent fingerprints when none of these differences is available. Concretely, it is difficult to visualize latent fingerprints when the following three conditions are all satisfied: (1) background fluorescence is much stronger, (2) wavelength range of background fluorescence includes that of fingerprints, and (3) fluorescence lifetime of background is close to or longer than that of fingerprints.

FLIM is a method of constructing an image whose pixel value is a fluorescence lifetime of the sample at that pixel position. FLIM is widely used in the field of life and biomedical sciences and, in forensic sciences, application of FLIM to the detection of gunshot residues is reported.<sup>2,3</sup> This study conducted FLIM on visualization of latent fingerprints for the case that the three conditions mentioned above are all satisfied.

An untreated fresh fingerprint was pressed on white, yellow, and pink paper. These samples were excited by a 532nm pulsed laser of 1mJ and pulse width of 3-5ns. Fluorescence images of samples were acquired with a fast-gated Intensified Charge-Coupled Device (ICCD) camera with a gate width of 2.8ns at the delay time of 6–20ns (1ns interval) to the laser pulse. The camera has a sensitivity to 200–850nm. The resolution of image is 1,024 x 1,024 pixels. A long-pass filter (>550nm) was placed between the sample and the camera. Fluorescence intensity and lifetime of each paper were as follows. Fluorescence intensity of white, yellow, and pink paper were about  $2 \times 10^2$ ,  $8 \times 10^2$ , and  $3 \times 10^4$  times larger than that of fingerprints, respectively. Fluorescence of pink paper was specifically strong. Fluorescence spectra of each paper covered the wavelength range of up to about 700nm and included that of fluorescence of fingerprints. Fluorescence lifetimes of each paper had a component of 5.9, 7.5, and 3.8ns, respectively, which were close to or longer than the fluorescence lifetime 3.7ns of fingerprints at 532nm excitation. In fluorescence images at any delay time, no fingerprint image was observed by the conventional time-resolved spectroscopy.

In spite of the largest fluorescence intensity of the pink paper by about four orders of magnitude larger than that of fingerprints, a latent fingerprint was visualized in the fluorescence lifetime image of the pink paper sample by FLIM. On the other hand, in white and yellow paper samples, fingerprints were not visualized in FLIM images. This seems to indicate that FLIM has the potential of enabling visualization of latent fingerprints on backgrounds emitting stronger fluorescence. It is shown that there is a case that FLIM visualizes latent fingerprints that cannot be visualized by the conventional time-resolved spectroscopy.

*This work was supported by JSPS KAKENHI Grant Numbers JP19K20687 and JP17K01385.*

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### Fingerprint, Fluorescence Lifetime Imaging, Time-Resolved Spectroscopy

## D46 A Simulation Triad for Evaluating the Use of Engineering Simulation

Bart Kemper, BSME\*, Kemper Engineering Services, Baton Rouge, LA

**Learning Overview:** After attending this presentation, attendees will understand key principles in using engineering simulations in support of forensic investigations, the legal pitfalls of simulation exclusion based on “simulations as a separate witness,” and how Verification & Validation (V&V) embodies best practices to connect the user’s skills to the simulation to enhance the likelihood given engineering simulations are admitted. This can be summed up as the proposed “Simulation Triad,” where one must establish two of the elements to seek a third. The three elements are theory, data, and simulation.

**Impact Statement:** This presentation will impact the forensic science community by mapping the legal principles that can result in the exclusion of simulations from testimony, enabling the practitioner to examine the work of others with a more critical eye as well as do the work to reduce the likelihood of a simulation being excluded.

The scientific method can be described as predicated on statistically analyzed experimentation for the falsification or validation of a null hypothesis. In forensic engineering, developing a controlled experiment may not be possible due to factors such as complexity, safety, or cost.<sup>1</sup> Perhaps a more fundamental difference is the scientific method is a way to investigate by testing a hypothesis, whereas engineering is about applying well-understood principles to achieve a predictable, repeatable outcome. Forensic engineering is often about applying these well-understood principles to model a theory regarding an event to predict a theoretical outcome or, conversely, to incorporate field data into these principles to test which physical models best match the data in order to develop a working theory of the event. The “theory” is defined as the sequence of events incorporating the applicable engineering principles, such as kinematics, heat transfer, Newtonian fluid flow, or strain energy failure theory.

Mathematical modeling can be done many ways, including simple calculations. As it becomes more complex and computers are incorporated, it can be termed a “simulation.” There are established numerical models such as Finite Element Analysis (FEA) and Computational Fluid Dynamics (CFD). The more complex the simulation is, the more potential there is for the workings of the simulation to be outside the individual’s personal control and understanding. This can result in the simulation being ruled to be a “separate witness,” which is not subject to cross-examination and is therefore excluded.<sup>2</sup>

While the concept of V&V is not new, it wasn’t until 2006 that a standard was developed for solid mechanics FEA, followed by CFD in 2009.<sup>3,4</sup> Other standards have emerged. Properly used, V&V techniques can be used as a method to meet a *Daubert* challenge, or what is now a Federal Rule 702 challenge. Specifically, it can address three of the elements of a typical Rule 702 challenge of assessing: (1) whether the simulation-related testimony is based on sufficient facts or data; (2) whether the simulation-related testimony is the product of reliable principles and methods; and (3) whether the expert has reliably applied the simulation principles and methods to the facts of the case.

Understanding the methodology proposed in this presentation will guide the simulation practitioner to best practices and increase the likelihood to meet a Rule 702 challenge as well as give those reviewing the work of others a method to evaluate simulation use.

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### Simulation, Verification, Validation

## D47 A Forensic Readiness Model for the Fused Filament Fabrication (FFF) -Based Additive Manufacturing (AM) Process

*Muhammad Rais, MS\*, Virginia Commonwealth University, Richmond, VA; Irfan Ahmed, PhD, Virginia Commonwealth University, Richmond, VA*

**Learning Overview:** This presentation will familiarize attendees with the challenges in the existing forensic readiness capability in Additive Manufacturing (AM) processes and the problems in employing conventional IT forensic readiness models in an AM process. Attendees will also understand the advantage of using physical domain artifacts in the forensic readiness models of Cyber-Physical Systems (CPS).

**Impact Statement:** This presentation will impact the forensic community in three ways: (1) readily using the model and the case study to achieve forensic readiness for FFF-based AM process, (2) providing guidance to create models for forensic readiness for CPS employing physical domain artifacts, and (3) urging forensic researchers in formalizing regulatory compliance criteria or the best practices for security-sensitive and critical 3D printing setups.

Currently, industrial manufacturing is going through a transformation phase of adopting additive manufacturing technology, which is fundamentally different from conventional subtractive manufacturing and offers a separate set of vulnerabilities and attack opportunities. For instance, malicious variations in the internal structure, anisotropy, or thermodynamic profile can cause inconspicuous damages during 3D printing. Another unique phenomenon of the merger of Information Technology and Operational Technology is happening concurrently in the industry, exposing the relatively isolated and less vulnerable manufacturing industry to the world of cyberattacks. In case of failure of a critical 3D-printed component (such as a blade of a jet-engine's turbine), a forensic investigation is held to find answers to questions such as: did the blade fail due to an attack on the manufacturing process chain, how the attack was conducted, which printing parameters were modified, etc. An important phase of digital forensics, known as forensic readiness, comprises pre-incident information acquisition of important information to help find answers to forensic questions during the post-incident investigation. Unless captured during the operation, some critical information may not be retrievable later during the investigation phase. For this reason, regulators produce compliance criteria and best practices for users to timely collect the potentially useful information and properly preserve it. As additive manufacturing is a cyber-physical process, its forensic readiness requirements are different from conventional IT infrastructure. The AM process chain does not terminate in the cyber-domain, urging the need to recover information from the later part of the chain in the physical domain. The physical domain's knowledge (if captured) can provide detailed information about the attack. The precious artifacts extracted from the physical domain can play a pivotal role in answering many forensic questions.

No work in the existing literature discusses the forensic readiness of additive manufacturing processes. To fill this void, this study presents a forensic readiness model for FFF-based 3D printing process that not only highlights the conventional cyber-domain information of interest but also extends the digital forensics reach to the physical domain by employing authentic methods to acquire the object's state throughout the printing process. The model utilizes multi-domain algorithms on the acquired printing data to answer the forensic questions. The approach readily and authentically extracts the cyber- and physical-domain artifacts, such as the G-code file (printing instruction file) in use at the time of the attack, the printer's firmware, the geometrical layout of each printed layer, and so on. Through a case study on a common printer, the effectiveness of the model is evaluated under most of the attack scenarios available in the existing literature. For instance, if an attacker modifies the thermodynamic profile of the printer's nozzle to induce undesired thermal stresses or creates tiny cavities at critical locations in an object causing premature failure, the proposed approach successfully finds traces of the attacks.

### Forensic Readiness, Additive Manufacturing, 3D Printers

## D48 Microscopy—Criminalistics and Environmental Forensic Applications

*James Millette, PhD\*, Millette Technical Consulting, Lilburn, GA*

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**Learning Overview:** From this presentation, attendees will learn how microscopy may be useful in the analysis of particles in forensic engineering investigations to obtain results like those determined for a criminal trace evidence examination.

**Impact Statement:** This presentation will broaden the use of microscopical analysis of particulate in engineering fields by using the forensic methodologies that have been developed for use in criminal investigations of trace evidence.

The Locard Exchange Principle was proposed in the 1930s by Edmund Locard, the director of the crime laboratory in Lyon, France. Locard's Exchange Principle states that whenever two objects come into contact, a transfer of material will occur. By recognizing, documenting, and examining the nature and extent of this evidentiary exchange, Locard observed that criminals could be associated with particular locations, items, or evidence and victims. Microscopy played an important role in finding the evidence of particle exchange in the Shannon Melendi criminal case in Atlanta in 2005. Eleven years after the abduction of the Emory University student, microscopical analysis using scanning electron microscopy found an unusual assemblage of metal particles (cobalt, molybdenum, titanium, and tungsten) on masking tape and in a cloth bag containing the victim's ring that had been left in a phone booth after an anonymous call had been made concerning the abduction. The same assemblage of metal particles was found on the same type of type in the vehicle of the suspect, Colvin (Butch) Hinton III, as well as at the Delta Airlines technical support center where he worked. This trace evidence of particle exchange helped to lead to a successful conviction of Mr. Hinton, even without a body or crime scene.

Similar microscopy techniques were used to identify the source of a child's high blood level when analyses of the paint in her home showed no evidence lead-based paint. Particles containing lead matching those found in the dust of the family home were found in piles of ash outside an abandoned warehouse down the street from the family's house. The warehouse property eventually became an environmental superfund site and the owner was found guilty of neglect.

In another case, microscopical analyses found a mixture of two asbestos types in pieces of cloth that had been taken to the family home from worksites by the father of a woman who had contracted mesothelioma. Even though the cloths had been used for drapes and rags, and washed several times, amosite and chrysotile asbestos fibers were found among the cotton fibers. The two asbestos types together were consistent with the composition of a product called Kaylo that was shipped to worksites packed in a cheesecloth-type material.

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### Microanalysis, Particles, Dust

## D49 Who Is a Good Boy? Finding Out What Makes a Performance Detection Dog

*Student Clément Martin, MS\*, Uliège, Gembloux, Namur, Belgium; François Verheggen, Uliège, Gembloux, Namur, Belgium; Claire Diederich, Unamur, Namur, Belgium*

**Learning Overview:** This presentation will allow attendees to understand the practices of professional Detection Dog's (DD's) handlers to scientific knowledge. Also, a definition of what is a high-performance DD will be proposed. Finally, an easy-to-replicate method will be described to assess DDs' performances.

**Impact Statement:** This presentation will suggest axes of research to better decipher DDs' physiologies and behaviors. It will also define for the first time the concept of DDs' performances based on a common agreement among professional police DDs' handlers. Finally, an accurate test allowing the assessment of DDs' performance will be described.

Dogs are known to be efficient scent detection animals. Their powerful olfactory system and their training ability explain why they are widely used in so many police and military application fields. However, low performance observed in DDs may result from a lack of knowledge on their olfactory neurophysiology and related behaviors.<sup>1,2</sup>

This work associated the results extracted from: (1) a deep review of the available literature, (2) an international survey sent to professional DD's handlers (n=50), and (3) an experimental assay performed on cadaver and drug DDs (n=20) to decipher what makes a performant DD and ultimately suggest a first definition of what is a high-performance DD.

The survey was sent to the police network for law enforcement dog professionals (Kynopol) and detailed answers were received from 50 cadaver DD teams. The questions were focused on the procedures of selection, training, and performance evaluation. Despite a lack of homogeneity in the selection methods used, selected DDs have usually the same physical and behavioral traits: the agility and the stamina of puppies were highlighted as the most important biological traits to pay attention to during the selection. Playfulness, curiosity, and sociability were also regularly pointed out in the answers received. Most surveyed handlers use reinforcement rather than punishment during their training sessions. Although a few teams practice negative reinforcement, the others use either toys, clickers, or encouragement as positive stimuli. Practitioners never use commercial training aids and prefer homemade biological aids that they declare being the most reliable. Both this survey and the literature review suggest that there is no existing definition of what a performant DD is, nor reliable test that could be used to evaluate post-training performance. Each team performs its own homemade method that is, in most cases, based on subjective measurements. Most of the surveyed DD teams define a performant DD as a dog which is confident, precise, with a high rate of success (low level of false negatives and positives, that is, high sensitivity and specificity of the detection) and which performs a strategic research.

Taking these results into account, this study set up a behavioral assay to assess DDs' performances in hope of validating this definition of a performant DD. Twenty dogs were assessed in a cinderblock half-circle arena (d=6m) in which a targeted odor was hidden among other distractive odors. A camera was placed on the ceiling (h=3m) and a video-tracking software was used to analyze the behavior, movement, and activity of the dog. This arena was validated based on co-inertia analysis between measured data in the arena and the dog evaluation by handlers. It allows the measurement of the dog's error rate and level of strategy.

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### Detection Dogs, Performance Assessment, Ethology

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## D50 An Evaluation of the Application of Data Fusion Technology to Sand Analysis

*Sergey Mamedov, PhD\*, HORIBA Scientific, Piscataway, NJ*

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**Learning Overview:** After attending this presentation, the attendees will have a better understanding of the application of Data Fusion technology to sand analysis, an essential part of forensic identification of these materials.

**Impact Statement:** This presentation will impact the forensic community by serving as critical aspects of sand analysis and an example of a practical application of the Data Fusion technology to materials identification.

This presentation aims to demonstrate the application of Data Fusion technology to classify sand from different sources. It will likewise show that Data Fusion technology can improve the results of such classification. This presentation will then compare and discuss the results of single source and data-fused analysis. X-Ray Fluorescence (XRF) is a valuable tool for the identification of substances and for confirming their identity with no sample preparation. XRF emission lines, specific to particular elements, enable elemental and chemical identification. XRF analysis has the potential to solve a wide variety of problems associated with forensic science. Statistical methods applied to a set of XRF spectra show the capability of detecting the slightest differences in the elemental composition of sand. Therefore, it plays a vital role in helping to determine the origin of sand. To illustrate the above, XRF spectra of sand will be highlighted in this presentation and the ability of XRF to identify sand from different geographic locations, including the United States, Europe, and the Middle East.

The XGT-900 XRF analytical microscope was used in this study. The XRF spectrum of the materials depends on the X-ray optical system, the detector's sensitivity, and accelerating voltage. In addition, the background from the substrate will contribute to the spectrum of the small pieces because excitation X-ray penetrates through the material and interacts with the substrate. Statistical data analysis such as Principal Component Analysis (PCA) or Partial Least Squares Discriminate Analysis (PLS-DA) takes these parameters into consideration.

This study collected and analyzed spectra of sand from different locations (the United States, Europe, and the Middle East) in the range of 1.00–40.96keV (<400 spectra). These spectra were used to build a data set for classification. The results show that PCA allows one to differentiate samples, which have very similar spectra features. To improve the accuracy of classification, the method of Data Fusion was applied to two sets of data. Examples of XRF spectra and trace element analysis of sand are presented, and the analysis results are compared and discussed. Data shows that the location of an unknown sample of sand may be predicted using PCA or/and Partial Least Squares Discriminant Analysis (PLSDA).

The combination of XRF and trace element analysis offers a powerful tool for the characterization and identification of sand. Pre-processing should be performed before concatenation of raw data. Classification of sand was improved using fused data. PCA model performed on fused data is more robust, and visual discrimination from class distributions is better than those results obtained by individual classification.

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**Sand Analysis, Data Fusion, XRF**

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## **E1 The Use of Smart Phone Applications in Crime Scene Analysis: Forensic Advantages and Limitations**

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**Learning Overview:** After attending this presentation, attendees will understand the role of smart phone applications in crime scene investigation.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the advantages and limitations of apps to perform measurements with respect to reference points placed on the scene.

Crime scene analysis requires a systematic method. First, the investigators delimit the scene and make measurements with respect to reference points using tools such as extendable or flexible meters. This method needs access to the scene using appropriate precautions to avoid contamination of the scene. In recent years, the technology for smart phones and tablets is spreading in the forensic field thanks to the development of applications, often available for free or at low cost.<sup>1,2</sup> To date, applications are available that take advantage of the smart phone's camera to measure the size of an object. The operator moves the camera, following the measurement direction until the endpoint. These applications allow also for the detection of the distance between two or more objects and place them in the surrounding space with respect to reference points.

This study used an application capable of taking measurements first in a test environment by measuring: (1) length, width, and depth of a single object; (2) distance between two or more objects; and (3) distance of two or more objects with respect to a reference point. The unit of measurement was the centimeter (cm) and the meter (m). Measurements were performed at a distance of 5 meters, then progressively nearer to the reference point. The same measurements were repeated varying the angle of the camera (90-45° to the right -0° center -45-90° to the left) and with different brightnesses. The measurements were photographed using the smart phone camera. The values obtained were compared with the real measurement or distance of the object using an extendable meter. Subsequently, the application was used in two forensic cases of judicial inspection in a closed environment. The investigators performed measurements of the distance of the corpse with respect to reference points. The data were analyzed and compared.

The use of smart phone apps has already been applied to measure elbow joint motions and to calculate the age of death and bloodstain age.<sup>3-5</sup> This study has shown that the various apps allow 3D measurements of the dimensions of objects. The greatest advantage of this type of application is that they measure the distance between multiple objects, thus placing them in the surrounding space quickly. However, the comparison of the data with respect to the test environment has shown that the measurements may vary due to the distance and angle of the camera.

In this case, the app made it possible to estimate the distance of the body with respect to the reference points before the investigator entered the room, therefore without the risk of contaminating the scene or dispersing traces.

The advantages of smart phone apps are: (1) 3D measurements of single objects; (2) knowing the distance between multiple objects or with respect to a reference point; (3) documenting the scene without contaminating it; (4) speed in measurements; (5) possibility of simultaneously taking photos with measurements; (6) they are low cost or free; (7) intuitive interface and the possibility of repeating photos; and (8) a quick transfer of photos by sharing app or PC.

The limits are: (1) variability of measurement depending on distance; (2) they do not work in the dark; (3) problems with the measurement of long distances; and (4) minor applications in open space.

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### **Forensic Science, Crime Scene Investigation, Smart Phone**



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## E2 Autopsy Findings in Hydrogen Sulfide Toxicity

*Robyn Parks, MD\**, Los Angeles County Department of Medical Examiner-Coroner, Los Angeles, CA; *Christopher Rogers, MD*, Los Angeles County Department of Medical Examiner-Coroner, Los Angeles, CA

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**Learning Overview:** After attending this presentation, attendees will understand the chemistry behind the production of hydrogen sulfide gas, along with the autopsy findings, including proper toxicologic examination, of hydrogen sulfide toxicity and be reminded of the importance of scene safety.

**Impact Statement:** The impact on the forensic science community includes highlighting the importance of scene safety during death investigation and reviewing a relatively uncommon method of suicide.

A 24 year-old healthy female was found unresponsive in her parked rental car outside the parking lot of a local recreation area by a deputy who was on routine patrol. As he approached the vehicle, he noticed a foul odor, became lightheaded, and observed a handwritten note in the window stating that the vehicle was toxic and to call the Hazardous Materials (HAZMAT) team. The HAZMAT team reported to the scene and observed chemicals mixed in a bucket in the passenger compartment. Upon entry into the vehicle, they identified multiple empty bottles of Lime Sulfur Dip® and The Works® toilet bowl cleaner within the car. A notebook within the vehicle contained a suicide note and a shopping list for the purchased chemicals.

Autopsy revealed gray-blue discoloration of the periorbital areas, mucosal surface of the lower lip, abdomen, and inner thighs. Internal examination revealed green-gray discoloration of the epiglottis, trachea, and mainstem bronchi with a moderate amount of frothy fluid noted in the upper and lower airways. Opening the cranial vault revealed the cerebrum and cerebellum to be diffusely gray in appearance. Coronal sectioning of the brain showed a striking accentuation of gray matter with a dark, olive green discoloration.

Toxicology performed on postmortem blood specimens revealed thiosulfate (18mcg/mL). Methemoglobin and sulfhemoglobin were requested but could not be performed due to specimen inadequacy. The cause of death was certified as hydrogen sulfide asphyxiation with the manner of death listed as suicide. Inhalation of hydrogen sulfide gas produced by mixing of chemicals was listed for the “how did injury occur” section.

Hydrogen sulfide (H<sub>2</sub>S) gas is a colorless gas that smells like rotten eggs. While traditionally encountered in workplace exposure to “sewer gas” or commercial manufacturing, it can also be created by mixing toilet bowl cleaner (containing hydrogen chloride acid as an active ingredient) with a sulfide-containing chemical (often sulfur tree spray). Inhalation of the gas can cause rapid, almost immediate death by inhibiting cytochrome oxidase leading to respiratory paralysis and coma.<sup>1</sup>

Autopsy findings are relatively non-specific, but may include evidence of irritation, including conjunctivitis, injected respiratory epithelium, and airway secretions. Additional findings may include green discoloration of the gray matter, cherry-red lividity, and dark brown blood. Toxicology should include thiosulfate levels, which is produced when exogenous sulfide is partially oxidized by hemoglobin and liver enzymes and can be measured with toxicological analysis.<sup>2</sup>

The oxidation of hemoglobin produces methemoglobin, which produces the dark brown appearance of blood noted upon phlebotomy. Sulfhemoglobin is not formed during the acute exposure to hydrogen sulfide and not only exists, in small amounts, in the body, but is also formed during the decomposition process.<sup>1</sup>

This case highlights the importance of scene safety for law enforcement, medical professionals, and medical examiner/coroner personnel, along with revealing the autopsy and toxicologic findings.

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### Hydrogen Sulfide, Suicide, Exposure

**E3 A Case of Skully: An Investigation of X-Files Proportion**

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**Learning Overview:** After attending this presentation, attendees will appreciate how rapid and convenient access to forensic resources at a possible death/crime scene can potentially save time and money. Attendees will also be reminded that each case must be approached with an open mind.

**Impact Statement:** This presentation will impact the forensic science and law enforcement community by highlighting the inefficient use of time taken from forensic anthropologists/forensic pathologists in examining remains that turn out to be non-human. A brief discussion of the potential for telecommunication in this situation will be introduced.

A skull was found by construction workers during the draining of a pond. Law enforcement officers remembered that a person had been reported missing in the area years prior. Law enforcement transported the skull to Wake Forest Baptist Regional Autopsy Center, which was three hours away. The skull arrived at the autopsy center covered in adherent mud, obscuring details of the skull. As the mud was gently washed away, curious details of the skull became more apparent. Examination of the teeth and an X-ray of the skull were consistent with non-human, non-organic remains. The quality of the skull reproduction suggested a 3D printer composite or a very detailed mold where sutures appeared to be carefully created, possibly with a Dremel® bit.

Coroners/medical examiners and forensic anthropologists often receive requests for analysis of bones or possible body parts that appear to be human of which the majority of these discoveries turn out to be non-human. Investigation still takes up valuable time, especially if the suspected bones/parts have to be transported to a forensic anthropologist or coroner/medical examiner's office that may be a lengthy distance away. Use of telecommunication would be a readily available solution in these situations.

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**Death Investigation, Forensic Anthropology, Artifacts in Forensics**

## E4 Medicolegal Investigation in Two Cases of Suspected Anticoagulant Overdose

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of a complete investigation to evaluate the role of medications in cases of anticoagulant users' deaths.

**Impact Statement:** This presentation will impact the forensic science community by underlying the need of combined clinical, laboratory, and toxicological approaches when evaluating a suspected case of anticoagulant overdose. The cases of two 80+-year-old patients under Vitamin K Antagonists (VKA) therapy with the suspicion of anticoagulant overdose will be presented.

**Case 1:** An 84-year-old woman taking warfarin for atrial fibrillation was admitted to the hospital for rectorrhagia. Laboratory tests revealed an International Normalized Ratio (INR) value >9 and high levels of creatinine and sodium, revealing a severe acute kidney injury. The patient died seven hours after admission. Autopsy revealed little hypostasis with evidence of rectorrhagia. Mucosal congestion involving the rectum and the distal colonic segment with bloody material in the lumen was found. Toxicological examination was not remarkable. The cause of death was due to a severe acute kidney failure.

**Case 2:** An 87-year-old man under acenocoumarol therapy for a prosthetic mitral valve was admitted to the emergency department with a history of diarrhea, drowsiness and altered consciousness with no evidence of recent head trauma. A Computed Tomography (CT) scan showed the presence of a subtle subarachnoid hemorrhage which, along with multiple bruises and a level of INR >20, which raised suspicion of an anticoagulant overdose. The patient died two hours after admission. Autopsy showed little hypostasis and extensive bruises. Severe bowel ischemia with melaena was found. Toxicological examination showed traces of acenocoumarol in the blood. The cause of death was due to multiorgan failure triggered by atherosclerosis-related mesenteric ischaemia.

VKA (acenocoumarol, warfarin) are widely prescribed for the prevention and treatment of thromboembolic complications of cardiovascular diseases.<sup>1</sup> Elderly and comorbid patients under anticoagulant therapy are at risk for anticoagulant overdose for altered kidney and liver function. According to an annual American report, 1,766 cases of exposure to prescription warfarin were observed and 84% of those accounted for unintentional overdose in 2014.<sup>2</sup> Bleeding is a frequent side effect of this treatment, and most of the risk factors in unintentional overdoses seem to be related to patients' lack of information and education.<sup>1</sup> In case of supra-therapeutic dosing, very high INR values are found. VKA overdoses are relatively rare. The majority of the cases are asymptomatic or with minor hemorrhagic symptoms mainly represented by gingival bleeding, and epistaxis. Major bleeding episodes include bleeding from the gastrointestinal tract, deep muscle hematoma, ecchymoses on the extremities, hematuria, intracerebral hematoma, and intra-alveolar bleeding.<sup>1,3-5</sup>

Considering that chronic anticoagulant users should be monitored weekly by evaluating the INR, supra-therapeutic dosing may raise the suspicion of a medical liability or, even worse, intentional overdose. In cases of sudden death without a clear cause and with evidence of bleeding, performing toxicological tests and a complete autopsic examination as well as an accurate evaluation of medical records are recommended. All of the findings must be interpreted with considerable caution to avoid misinterpretation in such cases that may, in the worst case, lead to a miscarriage of justice.

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### Anticoagulant Therapy, Overdose, Medical Liability

## E5 Sudden Cardiac Death in Myocardial Bridging

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**Learning Overview:** After attending this presentation, attendees will be aware that myocardial tunneling is a rare cause of sudden death and not always an incidental finding.

**Impact Statement:** Myocardial bridging as an etiologically specific cause of death is a diagnosis of exclusion in the face of grossly or microscopically demonstrable recent and/or remote cardiac ischemia in the anatomic territory of the particular bridged artery. The community will be able to understand the settings in which myocardial bridging should be considered as the cause of death.

**Background:** Myocardial bridging is a congenital coronary artery anomaly in which a coronary artery tunnels within the myocardium. While this anomaly can occur with any of the epicardial coronary arteries, myocardial bridges are usually found in the middle segment of the left anterior descending coronary artery.<sup>1</sup> Most patients with this condition are asymptomatic as it is generally considered benign.<sup>2</sup> At autopsy, it is oftentimes interpreted as incidental. One study revealed an overall prevalence of 19% (CI: 17-21%), with a prevalence of 42% (CI: 30-55%) in autopsy studies, 22% (CI: 18-25%) in CT studies, and 6% (CI: 5-8%) in coronary angiography.<sup>3</sup> Myocardial bridges are of clinical and pathological significance when they involve the left anterior descending coronary artery and have a long (20-30mm) and deep (3-5 mm) intramyocardial course.<sup>4</sup> When the condition is not benign, it can result in anginal symptoms, acute coronary syndrome, myocardial infarction, cardiac arrhythmias, and rarely, sudden death.<sup>1,2</sup> In such instances, contraction of the peri-vascular myocardium in systole causes occlusion of the blood flow in the artery through compression of the artery lumen.<sup>4,5</sup> Myocardial bridging as an etiologically specific cause of death is a diagnosis of exclusion in the face of grossly or microscopically demonstrable recent and/or remote cardiac ischemia in the anatomic territory of the particular bridged artery.

**Case Report:** Presented is the case of a 44-year-old Nigerian-American man with no past medical history who died due to myocardial bridging of the left anterior descending artery. The decedent was found by his wife dead at home in his bed. She had seen the decedent earlier in the morning and he had been in his normal state of health. He was last seen alive by his mother who visited in the early afternoon and left when he was asleep. When the wife was unable to wake him, she called emergency services and started resuscitative efforts. Emergency medical services arrived and attempted to resuscitate him but were unsuccessful. At autopsy, he had an unremarkable external examination apart from obesity (BMI 35.9kg/m<sup>2</sup>). On internal examination, he had slight hepatic steatosis and biventricular dilatation. Examination of the 260gm heart revealed myocardial bridging of the proximal-mid left anterior descending artery up to 0.5cm below the epicardial surface, for a span of 3.0cm. The coronary arteries had no atherosclerosis, including the bridged segment. The coronary ostia were normal in location and morphology. There was a focus of white fibrosis (0.2 x 0.2 x 0.2cm) within the subaortic septum. Similar slightly more yellow areas of pallor were noted at the superior aspects of the anterolateral left ventricular papillary muscle (2.0 x 0.7 x 0.7cm) and the posteromedial left ventricular papillary muscle. (1.0 x 0.4 x 0.4cm). The myocardium was otherwise homogeneous, dark red, firm, and free of hemorrhage or softening. The left and right ventricular walls were concentrically 1.1cm and 0.3cm in thickness, respectively, and the septum measured 1.3cm in thickness. The aortic valve had focal fenestrations. The endocardial surfaces and four cardiac valves were otherwise unremarkable and free of vegetations or thrombi. Microscopy revealed a focus of subepicardial acute interstitial hemorrhage within the anterior left ventricle, and focal interstitial, perivascular, and replacement fibrosis compatible with the foci seen grossly, which was highlighted by trichrome and reticulin stains. There was increased scattered interstitial fibrosis adjacent to intramyocardial left anterior descending artery and a few markedly hypertrophic myocytes associated with the areas of fibrosis. Toxicology was negative for drugs of common abuse. A cardiac-focused sudden death panel of 132 genes associated with various forms of cardiac channelopathy, non-cardiac channelopathy, and cardiomyopathy was negative.

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### Autopsy, Myocardial Tunneling, Myocardial Bridging

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## E6 Light Detection And Ranging (LiDAR) Sensor for 3D Reconstructions in the Autopsy Room

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**Learning Overview:** Through this presentation, attendees will learn that the LiDAR-sensor-integrated camera is a valid tool to introduce a sort of reproducibility into the autoptical practice.

**Impact Statement:** This presentation will highly impact the forensic science community because it shows the possible role of 3D modeling in the autoptical field. This work aims to promote the use of LiDAR-sensor-integrated cameras in the autopsy room.

Autopsy investigation is a complex and unrepeatable procedure. Therefore, it is essential to validate the autoptical findings for medicolegal purposes. Photography allows for the maintenance of archival data and to audit forensic practices. However, the quality and reliability of photographic records and autopsy reports are not always guaranteed.<sup>1</sup> Technical requirements and specific skills are indispensable to perform an adequate photographic report.<sup>2,3</sup> Moreover, 2D images could lead to distortion and data misinterpretation. In recent years, 3D reconstruction has supplemented medicolegal activities, mainly in crime scene reconstruction.<sup>4,5</sup> The introduction of photogrammetry has made 3D documentation easier than in the past. The required equipment consists basically of a camera and a computer.<sup>6</sup> However, this method also has some limits (e.g., the intrinsic variability related to the type of camera and the operator's skills). A LiDAR sensor is a remote sensing device that uses an infrared-light-pulsed laser to calculate the distance between two points. Those data could be combined with photographic information and used to construct 3D models of objects and surfaces. The arrangement of a LiDAR scanner next to the camera of some smart phones and tablets has made precise 3D models extremely manageable, even in the autopsy room.

Herein, 3D models of entire corpses, single body areas, and single organs obtained during different forensic autopsies are presented. The app TRNIO was first used, then data were processed by MeshLab. 3D models were qualitatively evaluated to assess their reliability in reviewing the autoptical procedure. 3D models were realistic, accurate, and allowed precise measurements. Smart phones and tablets have been demonstrated to be a reliable tool in auditing and reviewing the autoptical procedure, thanks to 3D technology. Furthermore, displaying 3D models as evidence in court would probably improve the effectiveness and understandability of medicolegal dissertation, demonstrating the reliability and manageability of 3D models in forensic practice.

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### Autopsy Record, 3D Modeling, LiDAR Sensor

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**E7 Enhancing Postmortem Care for Transgender Decedents: An Analysis of Global Violence and Forensic Methodologies**

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**Learning Overview:** Attendees will gain an understanding for the circumstances surrounding untimely deaths of transgender individuals globally and learn about the intersection of systemic violence, interpersonal violence, and postmortem analysis. Harm reduction strategies for forensic scientists will also be suggested.

**Impact Statement:** Transgender (trans) is an umbrella term utilized for individuals who identify as a gender other than the sex they were assigned at birth. Due to systemic and sociocultural biases against the trans community, these individuals face disproportionate rates of lethal violence. Since 1970, more than 4,200 deaths due to anti-transgender violence have been reported to the Transgender Day of Remembrance (TDOR) database across 75 countries. Unfortunately, these values likely under-represent the actual number of trans decedents within these parameters due to the lack of visibility offered to this community and the absence of standardized protocols for tracking this data. This under-reporting is further exacerbated by the lack of postmortem care received by trans decedents as forensic analysts typically assign a binary sex to a decedent based solely on external genitalia. Additionally, most medicolegal death records do not include a space to indicate gender identity, generating an added risk for inadequate postmortem care that may lead to individuals being misgendered, deadnamed, inaccurately identified, or unidentified. Many trans victims ultimately never receive justice simply because of their gender. These methodological flaws continue to feed the cycle of violence and marginalization experienced by the trans community.

Despite complications in tracking untimely deaths of trans individuals, TDOR has created a database using criminal reports, news articles, social media posts, petitions, and other documents to track trans decedents. This database offers extensive qualitative and quantitative information which provides insight into systemic and interpersonal violence, lethal risks, language usage, forensic methodologies, case status, cause of death, and circumstances around death for trans decedents. Thus, this presentation will utilize the TDOR database to inform the forensics community of the ongoing epidemic of trans violence and death, while also offering recommendations for harm reduction and enhanced postmortem care.

Circumstances and causes of death identified within this database are of forensic significance as they offer information crucial to decedent identification and completion of the death investigation. For example, of the thousands of cases recorded by TDOR, 84.3% of deaths have been categorized as violent, with most cases exhibiting blunt force, sharp force, gunshot wound, and/or thermal trauma. Violent deaths are followed by unknown causes of death (9.5%), suicides (4.1%), medical deaths (1.8%), and deaths in police custody (0.6%). Of those with known ages, the average age at death for trans individuals was 29.5 years, which is drastically lower than the global life expectancy of 72.6 years. The database also suggests that trans individuals within the Americas are the most at-risk geographically.

The disproportionate levels of interpersonal violence and lethal risk surrounding trans deaths indicates that forensic scientists must promote higher quality postmortem care for these individuals, as it is the responsibility of forensic analysts to reduce the harm done through death investigations. This presentation will offer a number of harm reduction strategies that should be adopted by all forensic scientists, such as including gender identity on medicolegal documentation, educating forensic analysts of gender, violence, and queer theory, standardizing data collection, modernizing language, and including trans individuals and advocacy groups in medicolegal training and death investigations.

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**Transgender Violence, Death Investigation, Human Rights**

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## E8 Suicide Rates and Trends in Allegheny County, Pennsylvania, During 2010–2019

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**Learning Overview:** After attending this presentation, attendees will recognize the importance medical examiners play in educating and promoting public health issues to their community, while additionally learning the relevance the cause of death plays in suicide research.

**Impact Statement:** This presentation will impact the forensic community by displaying the wide scope of information collected by medical examiners and the unique analysis that could be done to highlight rates and trends.

In 2017, the suicide rate for the state of Pennsylvania was 13.8 deaths per 100,000 residents, while at the same time, Allegheny County's suicide rate was 15.8 deaths per 100,000 residents.<sup>1</sup> Suicide studies and research are not a newly studied area and numerous research studies have established trends within the scope of suicide, more commonly examined with gender, age, and race. Though a less-examined demographic in this area of study is income level by location.

In this study, suicide data from the Allegheny Medical Examiner's office during the period of 2010 through 2019 was examined along with poverty threshold data from the Census Bureau's American Community Surveys. This research examined whether impoverished communities have higher suicide rates than non-impoverished communities. A follow-up question that was asked was: do impoverished communities have different rates and trends when examining cause of death in suicides?

In defining key aspects of this research, poverty was defined using the Census Bureau's poverty threshold. This measure was used because the poverty thresholds calculated by the Census Bureau are meant for statistical analysis.<sup>2</sup> The poverty threshold is then compared to the income threshold, with these values being assigned by family size and composition. An example would be a family of four comprised of two children under 18, would have the poverty threshold of \$26,246 in 2020.<sup>2</sup> With 130 municipalities, Allegheny County was selected, given the variety of different communities.

The percent of households living under the designate poverty threshold varied drastically between municipalities. Even neighboring communities showed stark differences. For example, one municipality had an average of 40% of households living under the poverty threshold, while a neighboring municipality had only 13%. It was also calculated that the fluctuating suicide rate across the timeframe was 7.55% to 12.51%.

This research makes a unique contribution by pairing data collected by the medical examiner's office with place-based data from the Census Bureau. Results from this study aim to improve education and preventative measures in different communities with higher rates of suicide. Examining suicide rates in coordination with location and income levels provides a macroscopic examination of suicide beyond examining individual cases to provide another layer of contextual information that can aid future prevention efforts.

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### Suicide, Income Threshold, Cause of Death

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## E9 The Recent Rise of Eutylone Intoxications in Onondaga County, New York

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**Learning Overview:** After attending this presentation, attendees will have a deeper knowledge of the characteristics of the popular emergent synthetic psychoactive stimulant commonly known as eutylone.

**Impact Statement:** This presentation will impact the forensic science community by presenting a review of cases and epidemiologic data focusing on the presence of eutylone in cases of death due to drug-related intoxication or toxicity.

Novel psychoactive substances have been rapidly emerging in recent years and are being identified by forensic laboratories worldwide. Eutylone is a novel psychoactive substance that is rapidly becoming one of the most popular synthetic stimulants. It is mostly found in powder form and mixed with other psychoactive substances, thus increasing the risk of severe or life-threatening complications.

A rise in the use of eutylone has been observed in Onondaga County in the recent period. In the present study, case files from the electronic database of the Onondaga County Medical Examiner's Office in Syracuse, NY, were reviewed from January 2020 to the present for drug toxin deaths in which eutylone was identified in postmortem samples. No limits regarding age, race, and sex were imposed. Only cases in which investigative and full toxicologic reports were available were included. Sixty-two subjects met the study criteria, 32 males and 30 females. Ages ranged from 20 to 69 with a mean of 38.8 years. Fifty-two subjects were listed as Caucasian, seven were African-American and three were other.

Recent experiments show that eutylone acts on the central nervous system in a similar way to cocaine, methamphetamine, and MDMA by binding monoamine transporters and inhibiting the reuptake of the neurotransmitters, dopamine, serotonin, and norepinephrine. The exact mechanism by which eutylone causes death is still unknown as many users mix eutylone with other substances and potentiators. Additionally, the current shortage of data concerning deaths related to eutylone prevents physiologic trends from being recognized, which may help in understanding its role in mortality.

This study will shed light on the rising trend of eutylone use and emphasizes the necessity of reporting deaths in which eutylone is present in toxicologic analysis in suspected overdose cases.

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### Eutylone, Intoxications, Drugs



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## E10 A Review of In-Custody Deaths at the West Tennessee Regional Forensic Center

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**Learning Overview:** This study presents a 5.5 year review of the in-custody deaths at the West Tennessee Regional Forensic Center (WTRFC) with breakdown of deaths by manner and cause.

**Impact Statement:** This presentation will address the most common manners and causes of death in in-custody and police-involved deaths in the majority of West Tennessee over a 5.5 year time period.

The goal of this study is to categorize in-custody and police involved deaths by cause and manner.

The Death Investigation and Decedent Information (DIDI) database, used by the WTRFC for documentation, was reviewed for all deaths recorded as in-custody, to include local, state, or federal prison and police-involved deaths from January 2016 to June 2021. The cases were then categorized by manner of death (homicide, suicide, accident, could not be determined, and natural) and subsequently subcategorized by cause of death.

The most common manner of death was natural at 48.6%, with the most common causes attributed to cardiovascular disease at 47.8% and infection at 26.7%. The etiologies of cases due to infection were as follows: viral, 50.0%; bacterial, 45.8%; and fungal, 4.2%.

Accident was the second-most common manner of death at 20.0%, with 91.9% of these deaths due to drug toxicity. Methamphetamine (35.3%), fentanyl (23.5%), or the combination of the two (38.2%) accounted for 97.0% of the overdose-related accidental deaths. Cocaine comprised 2.9% of these deaths. The remaining manners of death were as follows: suicide, 14.6%; could not be determined, 3.8%; and homicide, 3.2%. The demographic breakdown of cases was as follows: male, 94.1%; female, 5.9%; White, 53.0%; Black, 45.4%; and other, 1.6%.

The majority of deaths attributed to suicide took place in a single-person cell (85.2%); 11.1% occurred with others, and 3.7% were unknown. The majority of suicidal deaths were due to hanging at 92.6%. The ligatures used in hanging-related deaths were as follows: bed sheet, 80.0%; shoelace, 12.0%; unknown/information not available, 8.0%. Toxicology analysis showed a positive finding in 55.6% of cases with the remaining either having negative toxicology (33.3%) or toxicology testing was not performed (11.1%). Illicit substances were detected in 37.0% of the cases with positive toxicology results.

A total of 29 police-involved deaths occurred in the past 5.5 years. The majority of cases were determined to be homicides (82.8%), with the remaining cases reported as could not be determined (6.9%), suicide (3.4%), and currently pending final report (6.9%) with 93.1% of causes due to gunshot wound-related injuries. The purpose for an encounter with law enforcement was reported as 31.0% being due to a response to a non-domestic crime; 27.6% due to the attempted service of a warrant; 20.7% due to a response to a domestic dispute; 13.8% due to a response to a mental health call, and 6.9% currently undetermined at the time of abstract submission.

In-custody deaths encountered at the WTRFC during this 5.5-year review were predominately due to natural etiologies. Similar to the overall leading cause of death in the United States, cardiovascular disease was the most common natural etiology. The significant number of deaths either directly due to illicit substances, or where illicit substances were concurrently detected on postmortem toxicology, especially in suicidal deaths, is of particular interest in an in-custody setting. Future goals include evaluation of mental health histories, drug histories, and temporal relationship between date of incarceration and death. The majority of police-involved deaths occurred during a non-domestic crime.

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### In-Custody, Police-Involved, Incarceration

## E11 A Death in the Pool Is Not Just Drowning

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of complete postmortem examination in apparently simple cases to distinguish different types of sudden violent death.

**Impact Statement:** This presentation will impact the forensic science community by presenting the importance of complete postmortem examination in seemingly clear cases.

Diving into water, often by carelessly jumping head-first into water during recreational activities, can be very dangerous and cause severe spinal cord injuries. In these cases, mortality is generally due to head trauma with associated superior cervical trauma.<sup>1</sup>

The importance of postmortem investigations lies in the necessity to discriminate between death by head/cervical trauma and drowning. The impact following the dive generally occurs between the cranial vertex and a rigid surface, and it results in a minimal injury at the site with a contusion (ecchymosis/abrasion) that may go unnoticed. This results in a transmission of forces at distance that causes damage to the cervical district.<sup>2</sup>

Presented is the case of a young subject found dead in a pool who has undergone a medicolegal investigation. In this case, the cervical injury was associated with inhalation of gastric material resulting in the development of asphyxia “ab ingestis.”

During the site inspection, it was reported that the young subject got sick while he was in the pool. The witnesses had seen his body in the water, but no one reported the event’s dynamics. When authorities arrived, the body had already been moved outside the water. Food material was found beside the corpse. It also emerged from those present that the subject suffered from a non-specific cardiac anomaly.

The *public prosecutor* ordered that a medical investigation should be carried out. The autopsy revealed the presence of a bruising-excoriated area near the cranial vertex and a minute brain area infiltrated with blood in the parietal cortex. The unexpected finding was the presence of hemorrhagic infiltration in the pre-vertebral region; when these tissues were removed, a fracturative lesion of the cervical vertebrae C5-C6 was observed.

The spinal cord below these fractures presented sub-meningeal, peri-medullary, and posterior tissues blood infiltration. The airways and the branches were filled with gastric food material. The only cardiac anomaly found was the presence of a minute hole in the oval trench, devoid of pathological significance.

Radiological investigation of the C3-C6 vertebral segment taken during the autopsy showed a minute localized “tear drop” fracture at the level of the antero-inferior margin of the C5 vertebra body; C6’s body was completely fractured and divided into multiple bone fragments. Toxicology analyses were negative.

Histopathological investigations revealed focal peri-vascular hemorrhagic infiltration at the level of the parietal lobe, hemorrhagic infiltration of the dura mater with marginal involvement of nervous tissue, and a spinal root in the cervical cord segment. Meat and vegetable fibers in the medium and large-caliber airways were also found, with associated hyperdistention of distal airspace and alveolar edema outbreaks.

Death was attributed to acute respiratory failure resulting from obstruction of the airways by food material (ab ingestis) due to the cranio-cervical trauma. In the reconstruction of the dynamic, it has been postulated that the subject had impacted with his head on a rigid surface, with consequent cranio-cervical trauma and fractures in C5-C6 with medullary involvement, which induced a loss of consciousness and consequent onset of vomiting.<sup>3</sup> The loss of consciousness led to an alteration of the protective reflexes of the airways, failing that defense mechanism against liquids and/or solids entering into the airways.

In this singular case, the death is attributable to a combined mechanism consisting of cervical trauma, vertebral fractures, and spinal cord injuries associated with inhalation of food material resulting in respiratory failure from ab ingestis. Contrary to what was initially supposed, the hypothesis of death by drowning was excluded. Even in apparently clear cases, it is mandatory to perform an accurate medicolegal investigation in order to determine the certain cause of death.

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### Cranio-Cervical Trauma, Diving, Acute Ab Ingestis Asphyxia

**E12 Hanging: Between Doubts and Certainties**

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**Learning Overview:** After attending this presentation, attendees will understand the role of forensic investigation and autopsy in cases of hanging.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of circumstantial data and hanging marks in the evaluation of the dynamics of hanging.

Hanging is a form of death due to constriction of the neck when the force applied is derived from the gravitational drag of the victim's body weight. The most characteristic sign in death by hanging is the *hanging mark*. Characteristics of the hanging mark vary in relation to the type of suspensions and ligatures used.

This study describes a case series of hangings in which the hangings were doubtful or the means of suspension was not found around the neck of the corpse. The analysis of the suspension means used was conducted through the evaluation of the nature of ligatures: soft, smooth, hard, and rough. In addition, a detailed inspection was carried out with the analysis of the possible suspension means used by the victim and any reconstructions of the dynamics of the hanging with specific measurements. Finally, the compatibility of the analyzed means with the hanging mark was evaluated. In doubtful cases, the analysis of circumstantial data becomes fundamental and, in particular, the analysis of suicide notes, the analysis of the phones, and the social profiles of the victim through the application of the psychological autopsy method. Moreover, it is essential to analyze and compare all external lesions on the body and, in particular, the measurements of the possible means of suspension compatible with the lesions in the neck and the characteristics and course of the hanging mark.

The analysis of hanging marks allows us to assess the method of suspension used and to clarify the manner of death and the type of hanging. In particular, the forensic analysis of cases of hanging is not always easy, especially because, too often, despite being an asphyxiated death, an autopsy is not always requested and more and more often the scene is altered by rescuers or people who do not know how to move around the scene. Finally, when the dynamics with which the hanging took place are not clear, it is necessary to deepen the social and psychological context in which it took place in order to understand more than just the tip of the iceberg of a very widespread phenomenon.

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**Forensic Sciences, Hanging, Suicide**

### E13 Sudden Cardiac Death Related to COVID-19 in an Asymptomatic Teenager With a Negative Postmortem Swab

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**Learning Overview:** After attending this presentation, attendees will: (1) be aware of a rare case of sudden cardiac death in an asymptomatic young boy affected by COVID-19; (2) discover the possibility of detecting a SARS-CoV-2 infection in the case of negative postmortem swab; (3) understand the importance of a careful anamnesis in sudden deaths of young people.

**Impact Statement:** This presentation will impact the forensic science community by reporting a rare case of sudden cardiac death in an asymptomatic young subject affected by COVID-19 in which histological and immunohistochemical study were essential to identify SARS-CoV-2.

Clinical features of SARS-COV-2 infection are various; most affected people have mild or moderate symptoms and recover without hospitalization. The primary manifestation is acute pneumonia, but the SARS-COV-2 infection can also involve the heart.<sup>1,2</sup> Even though elderly people have the most severe prognosis, sometimes COVID-19 can affect young people with heavy consequences. This presentation reports a case of a young boy who died from a COVID-19 infection in Northern Italy.

The 14-year-old boy was found lifeless by his parents in his bedroom, laying on his bed. Hypostasis and rigor mortis were already evident. The first responder team could only confirm the death. Before this event, the young boy was in a good state of health; in fact, he practiced football at a competitive level, for which he was subjected to periodic cardiac controls with results being normal. The young boy was subjected to quarantine two months prior to his death because he had close contact with a relative who had tested positive for COVID-19. However, the young boy remained asymptomatic and was not subjected to any COVID-19 test.

A forensic autopsy was performed to clarify the cause of death. A postmortem antigenic nasal swab for COVID-19 was performed. Then, histological (hematoxylin eosin), immunohistochemical (using SARS-CoV-2 coronavirus nucleocapsid monoclonal antibody B46F), and toxicological investigations were performed. The autopsy was performed four days after death. The postmortem COVID-19 swab result was negative (it is important to note that there is no certain data in literature about the SARS-CoV-2 RNA detectability period in human bodies after death).<sup>3,4</sup> Conjunctival and cardiac petechiae were detected. Macroscopic lung examination revealed a weight of, respectively, 520g (right lung) and 510g (left lung); both lungs showed severe blood congestion and edema. Poly-visceral blood congestion was found. Tissue samples and biological fluids were collected during autopsy. The toxicological analysis gave negative results. The histological examination revealed interstitial pneumonia. A mild lymphocytic infiltrate was also observed in the heart interstitium, especially surrounding the conduction system. Considering the pandemic situation and the interstitial pneumonia observed microscopically, despite the postmortem COVID-19 swab being negative, a specific immunohistochemical staining using primary antibodies against SARS-CoV-2 was performed on lung and heart samples. Only the lungs tested positive.

Overall, the findings illustrated above allowed the medical examiner to identify the cause of death in a fatal arrhythmia due to extensive heart inflammation, very likely related to an asymptomatic SARS-CoV-2 infection characterized by interstitial pneumonia. SARS-CoV-2 could be responsible for several clinical conditions also affecting the heart, but there are only a few reports of sudden cardiac death in a completely asymptomatic subject or with limited pathological heart involvement.<sup>5</sup>

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#### COVID-19, Sudden Cardiac Death, Younger Individuals

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**E14 Older Adult Suicides in Clark County, Nevada (2017-2021): The Trends and Potential Impact of the COVID-19 Pandemic**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential impact(s) of the coronavirus (COVID-19) pandemic on older adult (>50 years) suicide trends.

**Impact Statement:** This presentation will impact the forensic science community by exploring whether observed trends and patterns of suicide among older adults has changed significantly since the beginning of the COVID-19 pandemic in March 2020.

The goal of this study was to assess the impact of the ongoing COVID-19 pandemic on older adults by exploring suicide trends in Clark County, NV, over the past five years. This investigation involved an analysis of differences in the rates at which suicides occurred, demographic trends, and causes of death before and after Nevada's initial emergency stay-at-home order was issued on March 12, 2020. It was hypothesized that stress and social isolation exacerbated by the pandemic may have contributed to an overall increase in older adult suicide deaths and that specific causes of death would differ among pre-pandemic and pandemic groups.

This preliminary sample included 997 adults aged 50 years and older whose manner of death was deemed a suicide by the Clark County Office of the Coroner and Medical Examiner (CCOCME) between the years 2017 and 2021. Ongoing research will include all older adult suicide deaths that occur until the end of this year. Older adult suicides occurring between January 2017 and February 2021 were analyzed as part of the pre-pandemic group in order to establish a more accurate baseline for the area. The data was then categorized into pre-pandemic (before March 12, 2021) and pandemic (after March 12, 2021) time periods and analyzed by demographic groups (i.e., sex, age, gender, race).

The data obtained from the CCOCME provided age, sex, and race demographics assigned by forensic staff as well as cause of death. This study focused upon trends among both older adults (>50 years) generally and older adults of geriatric age (65+ years) specifically. This was done in order to better understand the range of variation in suicide deaths among older adult age groups in regard to the pandemic. While not statistically different ( $p=0.19$ ), 44.7% of the pre-pandemic group were of geriatric age at time of death compared to 49% of the pandemic group. There were no significant differences in race and/or sex between the two time periods. Regarding cause of death, there was an increase in the use of firearms (57.1% to 67.7%,  $p=.002$ ) and a decrease in drug/alcohol-related suicides (19.2% to 10.8%,  $p=0.001$ ) in the pandemic period as compared to the pre-pandemic period, both of which were statistically significant.

It was expected that rates of older adult suicides occurring in Clark County would increase following the beginning of the COVID-19 pandemic, regardless of sex and/or race. Therefore, it was surprising that no statistically significant differences were observed for any demographic group between the pre-pandemic and pandemic time periods. The observed changes in cause of death categories were expected, although the specific categories in which the change would be observed (i.e., firearm and drug/alcohol) were unknown. It is possible that the observed changes in cause of death may be attributed to changes in what means of suicide were easily accessible. That being said, the rise in firearm deaths was not entirely surprising. More than 45 percent of households in Clark County own a firearm and so, as Nevadans began spending more time indoors, many may have had better access to weapons usually kept in the home. At the same time, accessibility to illicit drugs and/or alcohol may have decreased as people were quarantining due to the pandemic.

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**Public Health, Suicide, COVID-19**

## E15 Excess Deaths in Shelby County, Tennessee, During the COVID-19 Pandemic

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**Learning Overview:** Attendees can expect to learn the general trends in death throughout the COVID-19 pandemic, particularly focusing on those deaths unrelated to COVID-19 infection. Sociologic barriers to wellness will be explored as well as factors contributing to increases in violent and accidental deaths.

**Impact Statement:** Novel findings are presented on the impact of an unprecedented global health crisis on deaths in Shelby County, TN. The implications of various health and socioeconomic disparities on mortality will be discussed in the context of the COVID-19 pandemic.

COVID-19 has been the attributing cause of over 600,000 deaths in the United States to date.<sup>1</sup> However, all causes of mortality have shown a distinct increase outside of COVID-19-related deaths. During the year following the onset of COVID-19, the United States saw an overall mortality increase of 15.9%.<sup>2</sup> In 2020, approximately 3,358,814 deaths occurred in the United States; COVID-19 was listed as an underlying or contributing cause in only 377,883 (11.25%) of those deaths.<sup>2</sup> A similar trend was seen in Shelby County, representing a microcosm of the impact of COVID-19 on the nation.

From March 24, 2020, to March 24, 2021, total deaths in Shelby County increased from 3,591 to 4,620, a percent change of 28.65%. However, deaths due to COVID-19 infection represented only 37 of the additional 1,029 deaths (0.8%). The largest total increase of deaths was in the “natural” category, an increase of 545. This largely included deaths attributable to chronic diseases and complications of long-term health conditions such as cardiovascular disease, hypertension, kidney disease, COPD, cancer, and diabetes. Many patients with long-term health conditions were reluctant to seek medical care, fearing exposure to COVID-19 in health care facilities.<sup>3</sup> This could have exacerbated health complications that would otherwise have been treated, increasing the likelihood of fatality due to adverse events.

Deaths categorized as “accidents” in Shelby County increased from 851 to 1158, a 36.1% increase. Of these deaths, the largest contributors to accidental deaths were motor vehicle-related accidents and drug overdoses. Interestingly, despite the placement of stay-at-home orders, deaths due to motor vehicle accidents increased by 21.29%. This trend was seen throughout the nation, despite lower traffic counts, and could indicate an increase in risky driving behavior.<sup>4</sup> Even more astounding was an increase in drug overdoses: a 63.85% increase in overdoses followed the onset of the pandemic. These were largely attributed to opioid drugs (79.54%) such as heroin and fentanyl. This could be due to changes in drug supply chains, encouraging the use of more potent drugs like fentanyl. Additionally, increased drug usage due to feelings of isolation and a decrease in the availability of bystanders to intervene during an overdose could have contributed to the increase in drug-related fatalities.

Historic increases in national homicide rates accompanied the COVID-19 pandemic, with a 30% increase in overall homicides.<sup>5</sup> This trend was even more pronounced in Shelby County, with 40.88% more homicides compared to the previous year. Of particular note was a deeply troubling increase in gun violence. Gun-related homicides in Shelby County increased by 45.8%, compared to a national increase in gun assaults of 22%.<sup>5</sup>

At the onset of the pandemic, global uncertainty was compounded with isolation, causing many to anticipate an overwhelming increase in deaths of despair. Contrary to these expectations, national rates of suicide decreased from 2020-2021, even as local governments enforced social distancing measures.<sup>6</sup> These trends were paralleled in Shelby County: despite an increase in total number of deaths by 28%, the percent of deaths due to suicide decreased in the year following the onset of the COVID-19 pandemic. Suicides contributed to 2.79% of deaths compared to the 3.56% the previous year, representing a 21.67% reduction in risk of suicide during the COVID-19 pandemic.

Overall, trends in death rates following the onset of COVID-19 indicate the important role of social and economic unrest in violent and drug-related deaths as well as the importance of mental health support and accessibility to health care resources in promoting favorable health outcomes.

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### Excess Deaths, Epidemiology, COVID-19

## E16 COVID-19 Pandemic From the Forensic Standpoint: A Platform Against Future Biological Threats

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**Learning Overview:** After attending this presentation, attendees will understand that the scientific and experimental work found in the forensic field can be recognized and employed by the broader scientific community for solving particular problems. Attendees will learn how the contribution of the forensic institution on a multi-institutional SARS-CoV-2 project created the foundation for further development of forensic microbiology.

**Impact Statement:** The forensic science community will benefit from this growing-from-crisis experience because it well illustrates the need for flexibility, cooperation with other scientific fields, and accelerated adoption of technologically advanced procedures in forensics. This need is arising from increasingly complex challenges imposed on humanity by a rapidly changing global environment.

For almost a year and a half, the world has been struggling with challenges brought upon global society by the COVID-19 pandemic. The effort to adapt private and professional life conditions became an everyday routine for millions of individuals, businesses, and social entities alike. This study's governmental forensic DNA laboratory, in attempting to contribute to the COVID-19 response, has also experienced disruptions in forensic research activities, both in terms of human potential and infrastructure. Having recognized this lab's competences, academic partners included it in the nationally funded scientific project aimed at decoding the SARS-CoV-2 and COVID-19 host genetic sequencing.<sup>1</sup> Here is described the extent of the lab's contribution based on the internally validated protocol for Massively Parallel Sequencing (MPS) of the whole mitochondrial genomes.<sup>2</sup> Additionally, this study reports improvements of expertise on virus MPS by performing SARS-CoV-2 sequencing. Besides the evident social benefit from the collaboration of the national forensics laboratory with academia as a response to the immediate pandemic threat, this collaborative model opens up new aspects of future forensic research and development in the area of forensic microbiology.

MPS workflow of the Forensic Science Centre "Ivan Vučetić" Research DNA Laboratory is based on the Illumina® MiSeq® FGx® instrument for the whole mitochondrial DNA (mtDNA) sequencing from blood samples of COVID-19 patients (MiSeq® Reagent Kit v2 300cyc) and SARS-CoV-2 sequencing from patients' corresponding nasopharyngeal swabs (MiSeq® Reagent Kit v3 500cyc). Samples were collected and viral RNA was extracted (SaMag™ ViralNA Extraction Kit) and quantified (SARS-CoV-2 Real-Time) at the University Hospital Dubrava. Viral RNA was submitted to cDNA conversion (Quanti Tect® Reverse Transcription Kit) and library preparation (NEBNext® Ultra™ II DNA Library Prep Kit) at the Ruđer Bošković Institute, while the entire mtDNA workflow was conducted at the Forensic Science Centre "Ivan Vučetić" using Illumina® NexteraXT Library Prep Kit, as described previously.<sup>2</sup> Raw sequencing data were processed at the University of Zagreb, Faculty of Science. Sequences were mapped to reference genomes (either SARS-Cov-2 or human mtDNA) and variant calling was performed to determine viral mutations and mitochondrial haplogroups.

Ongoing project activities have so far resulted in optimization of the complex workflow, involving many researchers and institutions. Best results were obtained when nucleic acid samples were stored for less than 30 days after isolation and from individuals with the SARS-CoV-2 detection real-time Polymerase Chain Reaction (PCR) Ct values below 30. The molarity of viral sequencing libraries should exceed 5nM. Blood samples that were already used for the biochemical analyses are not compatible with mtDNA heteroplasmy analysis due to frequent cross-contamination, mandating the need for intact samples. For optimal sequencing cluster density, the molarity of SARS-CoV-2 libraries was increased from 12 to 20pM. For higher viral sequence coverage, PhiX control spike-in was decreased from 10% to 5%. Detected viral variants and their relative amounts followed patterns observed in neighboring countries and the rest of Europe (sequences available from GISAID and lineage assigned using PANGOLIN). Mitochondrial sequencing confirmed overall haplogroup distributions similar to that of the general population with, however, interestingly different haplogroup ratios between patient groups with mild and severe symptoms (fivefold higher proportion of macrohaplogroup K in patients with mild symptoms). Potential protective effect of any mitochondrial haplogroup should be further investigated in expanded patient pool.

Experience gained through this project is of huge benefit to the entire community, as multi-institutional effort in characterizing SARS-CoV-2 may be reflected in different forensic scenarios involving accidental or intentional biological agent release. We will therefore continue to strengthen and improve upon the established collaborative network and procedures beyond the current COVID-19 crisis, in order to minimize the harm from future biological incidents.

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### SARS-CoV-2 Sequencing, Forensic Microbiology, Mitochondrial Genome Sequencing

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**E17 Organizational, Adaptational, and Continuous Changes of the Forensic Sciences During the COVID-19 Pandemic: An Overview**

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**Learning Overview:** Following this presentation, attendees will understand how the restriction of the COVID-19 pandemic has affected forensic science.

**Impact Statement:** This presentation will impact the forensic community by providing up-to-date data about changes that the pandemic situation has imposed on forensic science approaches.

The COVID-19 pandemic had negatively impacted human life. To date, the predominant route of transmission of the SARS-CoV-2 is by air, through the droplets dissemination by virus-positive individuals. Nevertheless, there is evidence to support a risk of transmissibility of the infection that can also occur through contact with body fluids and tissues of COVID-19 subjects. Finally, the demonstrated persistence of the virus on different types of surfaces represents an additional source of risk. Given the characteristics associated with the transmissibility of the virus, forensic personnel have had to modify their actions in order to more effectively prevent the risk of SARS-CoV-2 infection.

Forensic science is defined as multidisciplinary, involving different expertise; the activities are very different, including crime scene investigation, external examination, autopsy, and genetic and toxicological examinations of tissues and/or biological fluids. At the same time, forensic operators may have direct contact with subjects in life, such as in the case of abuse victims (involving in some child cases), collecting biological samples from suspects, or visiting subjects in the case of medicolegal examinations. In similar cases, forensic operators are called on to implement methods to prevent the SARS-CoV-2 infection risk; particularly, a subject affected by SARS-CoV-2 infection could remain asymptomatic for 14 days or even more, before symptoms appear or, alternatively, remain asymptomatic.

This presentation aims to analyze how forensic personnel have modified their activities during the SARS-CoV-2 pandemic.

In forensic practices, all cases and relative specimens should be treated as potentially infectious. Several forensic organizations have demanded guidelines to perform forensic investigations and autopsies. The risks for operators become more evident in cases of DNA investigations, considering that the most used sample in order to obtain a DNA profile is the buccal swab. The sampling exposes the operator to a high risk of infection, so it is a procedure that should be performed with great care, using Personal Protective Equipment (PPE). Moreover, it has been suggested that all forensic laboratories should be classified as a Biosafety Level 2 (BSL-2) Laboratory, employing well-trained personnel, generating additional costs to guarantee biosafety practices and to modify the protocols used.

Finally, it is important to note that the forensic scenario is greatly changed: on the one hand, during the pandemic period the number of crimes has inevitably decreased (particularly for homicides and theft), on the other hand, it should be noted that medicolegal disputes have largely increased due to complaints about deaths that occurred in hospitals and in nursing homes related to COVID-19 disease.

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**Forensic Sciences, Multidisciplinary Approach, SARS-CoV-2**



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**E18 Determining Postmortem Intervals of Fetal Pigs Buried in Fayetteville, Tennessee, in June 2021 Using Attenuated Total Reflectance Fourier Transform Infrared Instrumentation Blood Analysis**

Catherine Walters, BS\*, University of Alabama at Birmingham, Birmingham, AL; Elizabeth Gardner, PhD, University of Alabama at Birmingham, Birmingham, AL

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the use of Fourier transform infrared instrumentation for analyzing extended decomposition.

**Impact Statement:** The presentation will impact the forensic science community by presenting an analysis of Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) instrumentation on extended decomposition in fetal pigs.

There are many factors used in determining the Postmortem Interval (PMI) in questioned deaths. For example, one measure is based on core temperatures of a dead subject, but core temperature is affected by many factors, including environmental temperatures. In addition, the visual guide for PMI is subjective regarding the stages of decomposition involving rigor mortis, algor mortis, and livor mortis.<sup>1</sup> Recently, researchers have been investigating ATR/FTIR to track the changes in blood as an objective measure for determining the PMI. Zhang et al. documented the changes in rabbit plasma and pericardial fluid by ATR/FTIR for the first 48 hours postmortem for 105 male white rabbits.<sup>2</sup> They identified several trends. The largest changes were associated with proteins within 1,700-1,500cm<sup>-1</sup>. The positively correlated loadings were in the antemortem group at 1,633cm<sup>-1</sup> and 1,515cm<sup>-1</sup>. The negative loadings characteristic of increasing PMI included 1,650cm<sup>-1</sup> and 1,547cm<sup>-1</sup>.<sup>2</sup>

This study focuses on the extended period of PMI of fetal pigs after approximately three days postmortem. Fetal pigs were chosen due to the similar composition of tissue to that of the human species.

Six frozen fetal pigs were thawed three days postmortem and then were buried in Fayetteville, TN. Samples of blood were taken at approximately 72 hours, 4 days, 5 days, and 6 days at peripheral and umbilical sites. The samples were analyzed using the Perkin Elmer Spectrum 100 FTIR with a zinc selenium ATR attachment with 16 scans. Both changes in peak intensity and shifts in peak wavelength were analyzed for 3,280cm<sup>-1</sup>, 2,940cm<sup>-1</sup>, and 1,400cm<sup>-1</sup>. Several trends were identified for the extended periods of analysis between the six pigs. The intensity of the amides I's and amide II's peaks decreased in the span between 72 hours and 4 days in all specimens. However, for the remaining trends, at least one of the six pigs did not follow the pattern. Previous studies reported only the averages of the FTIR spectra, but this research showed variances in the data that indicated the average would not give a reliable representation in all cases samples.

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**PMI, ATR/FTIR, Decomposition**

## **E19 Equine Scent Detection in Search and Rescue and Search and Recovery**

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**Learning Overview:** After attending this presentation, attendees will understand the concept of equine scent detection, how it can benefit search and rescue (air scent) and search and recovery (human remains detection), and how it can be utilized by search and rescue organizations, medical examiner offices, and law enforcement agencies.

**Impact Statement:** This presentation will discuss the concept of equine scent detection, how it can be utilized in search and rescue and search and recovery missions, and how it can benefit both medical examiner offices and law enforcement agencies in finding and recovering human remains.

In 2021, Highlands Search and Rescue (HISAR) was created by members of various Maine Association of Search and Rescue (MASAR) personnel. The team consists of both Ground Searchers and Mounted Searchers. The President and one Vice President of HISAR are both in training to be certified in equine air scent, which involves searching for missing persons who are assumed to be alive. There is one member who is a Mounted Trainee. All members of HISAR and any MASAR team must be certified through MASAR as a Search Team Member and can obtain additional training that will benefit the SAR team.

HISAR has developed the first Mounted Air Scent Team Certification and is in the processes of making revisions.

SAR canines (K-9s) are the typical animal resource that is used and requested for locating missing persons because of their scenting abilities. K-9s are able to use air scent, cadaver scent (**Human Remains Detection** [HRD]), narcotics, ballistics, explosives, invasive species, COVID-19, etc. Horses also can use their olfactory skills to locate humans who are lost; they can also detect human remains and narcotics. There are very few organizations in the United States that train and deploy air scent and HRD horses, one which is led by Terry Nowacki, who has conducted clinics and trainings in Minnesota that HISAR Mounted team members have attended.

Although equine olfactory skills differ from K-9s, some researchers feel equine scent skills can be equal to, or greater than, that of K-9s. Even with domesticated horses, they must utilize their sense of smell daily; they must be able to find safe drinking water, safe food sources, mates, their offspring, and must always be aware of their surroundings as they are prey animals. Horses are unable to see objects directly in front of them and depend on the sense of smell. Horses have large flexible nostrils that can pull in large amounts of scent; when they sniff large amounts of scent, the odor is passed into nasal passages, allowing the receptor cells and the odor molecules time to interact and thus giving the horse time to evaluate the odor. Horses can scent on the ground or up to eight feet in the air. Horses are also able to cover a large search area, and having a rider atop the horse can give a different view than that of a ground searcher, K-9, or air searcher.

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### **Equine Scent Detection, Human Remains Detection, Equine Search and Rescue**

## E20 Insect Activity During Decomposition in the Extreme Sonoran Desert Climate

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**Learning Overview:** The primary goal of this presentation is to describe insect activity patterns observed during decomposition in an extreme environment in the southwestern United States, specifically related to an investigation of methods used to estimate postmortem interval.

**Impact Statement:** This presentation will impact the forensic science community by reporting novel observations of insect activity made during a study of decomposition in a desert environment, contributing to the ongoing efforts of anthropologists, entomologists, and death investigators in documenting the postmortem period.

The Sonoran Desert, which covers a large portion of the southwestern United States and northern Mexico, is a unique environment with extreme fluctuations in temperature and humidity, even in a single season. During the summer months, the temperature averages 104°F (40°C) but can reach 118°F (48°C).<sup>1</sup> In a single day, the temperature can also drop as much as 59°F (15°C) overnight due to the lack of moisture and low vegetation.<sup>1</sup> During the summer, the Ultraviolet (UV) index averages in the 9-10 range of a scale from 0-11.<sup>2</sup> The UV index represents a measure of the intensity of the sun's UV radiation, where a higher number represents greater exposure. This extreme climate represents a stark deviation from the other geographic contexts where decomposition stages and rates have been studied extensively, and little is known about the resulting variation in macroscopic changes to the body or associated insect and predator activity.

Presented here are observations of insect activity made during a study of decomposition of porcine models in a period of extreme temperature fluctuations. During the summer of 2020, two porcine models were clothed and placed in observation cages in natural desert settings outside of Phoenix, AZ, as a single phase of an ongoing longitudinal study investigating the timeline and stages of decomposition in this climate. One model was placed in direct sunlight while the other was positioned in the shade of a cluster of Palo Verde trees. Photos were collected in 30-minute intervals, and temperature and humidity readings were collected every 10 minutes from June 28, 2020, to July 22, 2020.

Insect activity during decomposition is well documented in a number of geographic zones, but little research has been published investigating the effects of an extreme desert climate on insect activity during decomposition. Studies representing other climates have concluded that heat allows insect colonies to thrive, though extreme spikes in heat were determined to be the cause of insect deaths, which slowed the active decomposition.<sup>3</sup> Insects have also been documented as showing signs of heat stress, resulting in a large number of deceased maggots on porcine carcasses left in the sun.<sup>4</sup>

This study's observations of insect activity differed from these reports. Despite differential sun exposure, the temperature ranges throughout the period observed were consistently similar for the two porcine models. However, the insect activity on each model exhibited observable differences. Within the first week of the study, maggots were active throughout the entire day on the model in the shade, while they were only active at sunrise and after sunset on the sun-exposed model. This study's observations suggest that temperature alone does not seem to drive maggot activity period in this environment, as both populations were exposed to similar heat. The apparent correlation between sun exposure and insect activity—not between heat and activity—will be explored in future studies through the collection of further measurements, especially the UV index in each location. This project represents the first phase in a long-term study that aims to bring clarity to these observations and to our understanding of the complicated patterning of decomposition in the Sonoran Desert.

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### Decomposition, Extreme Climates, Insect Activity

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## E21 Forensic Species Identification Using Phylogenetic Proteomics

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the forensic application of phylogenetic proteomics to wildlife crimes.

**Impact Statement:** The ability to categorically identify fur-bearing species, in the absence of detectable DNA, has the potential to fill a sizable gap in forensic monitoring and law enforcement of wildlife crimes.

The distribution of illegal fur products presents a major challenge for wildlife forensics and law enforcement. Processed fur is a major component of illegal trade. Illegal traffic in fur promotes and enhances other illegal commercial networks, including illicit drugs, human trafficking, and other environmental crime. Any policy from law enforcement and agencies tasked with combatting wildlife crime requires forensic tools to both positively identify a species; but also to exclude closely related, often legal, sister species. Unfortunately, modern industrial fur processing effectively removes DNA from the sample, depriving investigators of the major tool to identify listed and protected species. Even when DNA is available, this can be a challenge.<sup>1</sup> When DNA is absent, forensic investigators are forced to rely on morphological analysis that is time consuming, requires access to a limited number of highly trained individuals, and can have poor species resolution.

Phylogenetic information, however, can also be obtained from animal proteins, including proteins in animal hair and skin. With evolutionary biodistance amino acid sequence variants, single amino acid polymorphisms will become major alleles, then population-specific markers, and eventually species-specific markers. Over evolutionary time, and under the constraints of selective pressure and genetic drift, these accumulate and equivalent genes become less homologous. Genetic changes at the DNA level are also reflected by changes at the protein level, with species-specific changes accumulating in the amino acid sequence. As evolutionary distance increases, the proportion of common sequences and peptides between species is reduced. This can be used to identify unknown species. The mass spectrometry fragmentation spectra from the fur proteome will better match with protein sequences from the same or related species, giving great protein coverage.

To demonstrate this concept, this study processed hairs from a lion paw confiscated during law enforcement. Single hairs were processed using an optimized method for human hair and applied to a Q-Exactive™ mass spectrometer.<sup>2</sup> The resulting dataset was searched using the PEAKs peptide spectra matching algorithm and the closely related Leopard reference proteome. For single hairs,  $1,020 \pm 110$  (av  $\pm$  SD) peptides were identified ( $n=3$ ). The number of identified peptides was reduced with evolutionary distance. The more closely related leopard and tiger protein sequences, part of the *Panthera* genus, performed better than the less-related Cheetah proteome that was part of the *Acinonyx* genus. Hair-specific keratin proteins such as keratin 35, keratin 36, keratin 38, and keratin 75 all saw patterns of reducing alignment between spectra and protein sequences for leopard, tiger, then cheetah protein sequences. For example, the later protein, keratin 75, observed a protein coverage of 48%, 42%, and 39% when using the leopard, tiger, and cheetah proteomes respectively.

This demonstrates that protein coverage is a good surrogate for evolutionary distance, and that proteomics have the potential to identify species from single hairs from fur samples. With more information, this may be extended to differentiate different species and exclude sister species. The ability to categorically identify fur-bearing species in an automated manner and in the absence of detectable DNA should fill a sizable gap in forensic monitoring and law enforcement of wildlife crime.

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### Proteomics, Processed Fur, Environmental Crime

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## E22 Forensic and Investigative Scene Factors Associated With the Undetermined Manner of Death

*Betsy Adelizzi, PhD\*, American Military University, Charles Town, WV*

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**Learning Overview:** This research examined the forensic and investigative death scene field factors, in addition to the victim circumstances associated with the undetermined manner of death, through the lived experiences and insights of death investigators.

**Impact Statement:** The investigation, scene, victim, and investigator all serve as unique variables for understanding the patterns related to corroborating and excluding evidentiary information for the manner-of-death determination. Internal and external case resources can inhibit or help investigators reduce undetermined cases; medicolegal investigator training and certification standards were reported as inconsistent, and the lack of scene response was noted as hindering a comprehensive investigation; infant deaths, drug overdoses, and decomposed cases were susceptible to a greater risk of undetermined findings; both detailed and accurate death scene field factors and victim circumstances are necessary to reduce ambiguous manner-of-death findings; and gaps and problems still exist related to an undetermined manner of death.

Undetermined deaths in the United States are on the rise and correlated to misclassified suicides, accidental deaths, overlooked findings, and inaccurate outcomes within the criminal justice system. The undetermined category has not been separately assessed within the different types of medicolegal investigative agencies. This research examined the forensic and investigative death scene field factors, in addition to the victim circumstances associated with the undetermined manner of death, through the lived experiences and insights of death investigators. Two research questions were created to help understand the undetermined manner of death phenomenon: (1) What are the perceptions of study respondents regarding how examining the forensic investigative death scene field factors help reduce the undetermined manner of death findings?; and (2) What are the perceptions of study respondents regarding how examining the forensic investigative death scene victim circumstances help reduce the undetermined manner of death findings?

A qualitative case study was conducted by triangulating data with professional interviews, short answer surveys, and secondary data review. Coroner and medical examiner officials who certified death certificates and oversaw death investigations were interviewed. Death investigators submitted short answer survey feedback. Secondary data within the National Violent Death Reporting System (NVDRS) was also used. Thematic analysis identified patterns related to answer the research questions. Secondary data findings identified an increased rate of substance abuse and mental health problems, alcohol dependence, physical problems, and recent crisis as indicators for the undetermined manner. The investigation, scene, victim, and investigator all served as unique variables for understanding the patterns related to corroborating and excluding evidentiary information for the manner of death determination. Participant findings included the following: internal and external case resources can inhibit or help investigators reduce undetermined cases; medicolegal investigator training and certification standards were reported as inconsistent, and the lack of scene response was noted as hindering a comprehensive investigation; infant deaths, drug overdose, and decomposed cases were susceptible to a greater risk of undetermined findings; both detailed and accurate death scene field factors and victim circumstances are necessary to reduce ambiguous manner-of-death findings; and, gaps and problems still exist related to an undetermined manner of death. Further research into all sizes, designs, and jurisdictions for undetermined outcomes to better understand gaps, complexities, applications, and knowledge from those practices was recommended.

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### Medicolegal Death Investigation, Undetermined, Scene Investigation

**E23 “Our Hearts Are Not at Rest”: A Critical Look at the Adequacy of Indigenous Death Investigations**

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**Learning Overview:** Attendees can expect to learn how death investigation procedures conducted by law enforcement and medicolegal practitioners can be inadequate for Indigenous cases. This research will reveal common misconduct in Indigenous death investigations, express the need for decolonization in standard practices, and suggest alternative procedures that may best support the Indigenous community.

**Impact Statement:** The aim of this research is to teach death investigation practitioners how their practices may negatively impact Indigenous communities. The intention is to provide the forensic community with both the tools to start questioning colonial assumptions embedded in standard practices and suggestions for how to modify their approaches to better support future Indigenous cases.

Across Canada, thousands of Indigenous testimonies have reported deficiencies in how police and medicolegal professionals investigate their deaths. The problem, however, is that these individuals are summarily and systematically denied the resources to challenge investigators, and there are few cases that have done so successfully. This research establishes a comparative model to examine whether case investigation conduct aligns with standard investigative practice requirements. The qualitative sample includes three Indigenous case studies involving the suspicious deaths of young individuals in Prince Rupert, BC. The results present a central theme of inadequacy across all three cases, primarily in improper evidence collection, limited procedural follow-through, and withheld information. The outcomes of this study suggest a need for: (1) future research, (2) professional action to create and uphold effective accountability measures for investigators, and (3) a critical look into how colonial powers limit investigative effectiveness for Indigenous deaths.

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**Indigenous, Death Investigation, Best Practice**

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## E24 Forensic Science Data Integration and Stakeholder Workflow for Medicolegal Death Investigation Systems

*Matthew Gamette, MS\*, Idaho State Police Forensic Services, Meridian, ID; Jeri Roper-Miller, PhD, RTI International, Research Triangle Park, NC*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the data sharing and workflow processes among stakeholders of the medicolegal death investigation system.

**Impact Statement:** This presentation will impact the forensic science community by presenting the findings of a working group focusing on forensic sciences data exchange and stakeholder workflow processes for medicolegal death investigation systems. This presentation specifically describes data exchange needs and processes to seek improved data sharing methods, standardize data, and common language utilization for the medicolegal death investigation system.

The National Institute of Justice (NIJ), in partnership with its Forensic Technology Center of Excellence (FTCoE, 2016-MU-DX-K110) at RTI International and the Center for Disease Control and Prevention (CDC) convened a virtual Medicolegal Death Investigation Data Exchange Working Group (MDI-DATA-WG) over a 12-month period beginning in September 2020. The MDI-DATA-WG was formed to advance forensic science and ensure communication between Medical Examiners and Coroners (ME/C), death investigators, forensic scientists, and other stakeholders.

The objective of the MDI-DATA-WG was to identify data commonly collected by ME/C, data relied on by ME/C to make cause- and manner-of-death determinations, and data exchanged across the forensic science disciplines collection as it informs data integration processes. Particularly, the MDI-DATA-WG aimed to explain the ideal state of the MDI data exchange among data consumers and producers and the data types and systems they use.

The MDI-DATA-WG of more than 40 stakeholders (e.g., data producers like ME/C, data consumers like federal agencies) participated and further formed Subcommittees (SC) that focused on a specific purpose and goals. The MDI-DATA-WG met in five two-day meetings and intermittent SC monthly meetings. Approaches to collecting information included user case scenarios and feedback stakeholder types; presentations from invited guests with Q&A sessions; group experiential sharing; and forums with data programmers and technology developers. The work of the MDI-DATA-WG resulted in a final report published by NIJ's Forensic Technology Center of Excellence (FTCOE) and Centers for Disease Control and Prevention (CDC) to include guidance to move toward consistency, data standards, and best practice for improving the process. The efforts of this working group will result in various user case profiles, presentations, implementation forums, further research, and process mapping and evaluations of the various processes.

This presentation will focus on the work of one subcommittee directed on data types, data exchange systems (e.g., health information exchange systems), stakeholder data flow process relationships, and needs to improve upon the data exchange in an ME/C data system, as well as potential futuristic practices for collection. The MDI-DATA-WG produced guidance for what ME/C computerized information management systems should collect and archive. Other information collected includes various data sources available to pathologists from public safety, public health, and forensic science service providers, including information concerning initial response calls, police incident data, laboratory testing results, and information from first responders. The working group provided recommendations for drug terminology and ideas for how to network ME/C with other real-time data sources.

The demand to further understand and communicate data exchange needs and processes is critical for process improvements, to standardize data, and common language implementation for the MDI system. Stakeholder-informed data collection helps to address the opportunities and challenges for automated approaches and best practices to use and exchange data between medical examiners, coroners, forensic toxicologists, and other stakeholders. Additionally, next steps and observations for further research and resources to improve data sharing are presented and documented in a final report that is available on the FTCOE website ([www.forensicCOE.org](http://www.forensicCOE.org)).

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### Medicolegal Death Investigation Systems, Data Exchange, Data Sharing

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## **E25 Medicolegal Systems and Forensic Services Assessments: Methods to Better Understand the Needs and Challenges of Providing Structured Support and Building Sustainable Forensic Capacities**

*A. Skylar Joseph, MS\*1, International Committee of the Red Cross, Geneva, Switzerland; Maria Dolores Morcillo Mendez, MD, International Committee of the Red Cross, Geneva, Switzerland; Pierre Guyomarc'h, PhD, International Committee of the Red Cross, Geneva, Switzerland; Denise Abboud, PhD, International Committee of the Red Cross, Amman, Jordan; Eliane El Hajj, MBA, International Committee of the Red Cross, Beirut, Lebanon; Rawan Chaaban, MPH, International Committee of the Red Cross, Beirut, Lebanon; Myriam El Helou, BS, International Committee of the Red Cross, Beirut, Lebanon; Fondebrider, PhD, International Committee of the Red Cross, Geneva, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will have learned about the importance of medicolegal system assessments and a comprehensive tool developed for this purpose, which considers aspects beyond technical capacities. This presentation will focus on how medicolegal system assessments have allowed the International Committee of the Red Cross (ICRC) to develop and implement more structured strategies and activities to support affected populations, local authorities, and forensic practitioners as well as to build sustainable local capacities in the contexts the ICRC works in around the world.

**Impact Statement:** This presentation will impact the forensic science community by highlighting useful resources currently being utilized by the ICRC to assist in strengthening medicolegal systems and building sustainable forensic capacities around the world.

In the framework of its humanitarian mandate, the ICRC highlights the importance of the medicolegal system and forensic institutions to prevent and respond to humanitarian needs of people affected by armed conflict, emergencies, and other situations of violence. A reliable, well-functioning medicolegal system is essential both for a properly functioning justice system and contributing to prevention and protection in the social, legal, and humanitarian spheres. Forensic expertise and the proper use of forensic services by the judiciary help ensure adequate medicolegal investigations of suspicious deaths, including the necessary documentation and preservation of evidence required for investigation processes. This ensures that the dead and their families are treated with dignity and respect, to the best standards possible, and that bodies are identified and have a dignified final disposition. When searching for missing persons, the medicolegal system plays an important role in ensuring adequate integrated processes, proper involvement from families, and the provision of information. In the case of large-scale events, such as on-going/post-conflict situations, natural or man-made disasters, migration, and/or other emergency situations, the medicolegal system and forensic services must coordinate with other entities to ensure an efficient response.

Moreover, following events involving a rapid surge in the number of separated, missing, and/or deceased, few countries have the economic and structural capacities to properly respond. Even locations with well-developed medicolegal systems and advanced forensic facilities may become overwhelmed by the magnitude or complexity of these situations. This is especially so where the affected area lacks a pre-established or well-implemented emergency preparedness and response plan that entails the required actions toward the management of the dead and their families.

When the ICRC forensic unit is asked to assist local authorities and practitioners in their response to humanitarian needs, conducting a comprehensive forensic country assessment is key to understanding the current situation, identifying gaps in the current response, discovering the needs, limitations, and root causes, as well as assessing the capacities and working conditions of different actors. It also promotes better involvement of families in the search for missing persons and engagement with the relevant authorities. This process aids in determining context-specific priorities, developing strategies and programs, and identifying the needs of the affected populations, the communities, and the existing entities of the medicolegal system.

To achieve this, the medicolegal system assessment tool was developed to take into consideration the structural aspects of the judicial and investigative agencies, the technical capacities, while also analyzing the particular context, including existing political, economic, social, cultural/religious, and humanitarian settings. It enables ICRC forensic field staff to respond to changing environments and build upon the interventions of their predecessors through a clearer documentation process. This enables the development of an adequate Country Forensic Program that aligns with the specific context and needs, focused on three main humanitarian issues: (1) protection of and respect for the dead and their families in all circumstances; (2) preventing and resolving missing persons and ensuring support to their families; and (3) supporting the living victims of ill treatment, sexual violence, and other situations of violence.

Humanitarian and development organizations aim to support affected populations in various ways; it is often best to promote and implement programs that center community engagement, local partnerships, and that ultimately are aimed at building local capacities while being considerate of local religious/cultural practices and any political sensitivities. These types of considerations are essential in order to properly support the entities responsible for the protection and management of the dead and their families, through the clarification of the fate and whereabouts of missing persons from all circumstances.

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### **Humanitarian Forensic Action, Medicolegal Systems, Protection of the Dead**



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## E26 Addressing Emerging Humanitarian Challenges Through an Innovative Concept: The International Centre for Humanitarian Forensics (ICHF)

*Melahat Elif Gunce Eskikoy, PhD\*, ICRC, New Delhi, India; Govindarajulu Rajesh Babu, PhD, NFSU, Gandhinagar, Gujarat, India; Darshana Ishwarbhai Sharma, PhD, ICRC, New Delhi, India; Abraham Johnson, PhD, NFSU, Gandhinagar, Gujarat, India*

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**Learning Overview:** A meaningful novel concept integrating operations and academia in the field of Humanitarian Forensic Action (HFA) will be presented.

**Impact Statement:** Institutionalizing HFA within an existing university system can amplify available multidisciplinary expertise and existing technology and create regional and global synergies shaping the future of humanitarian forensic action.

In the past few decades, forensic science and forensic medicine methodologies and principles, employed in humanitarian settings, helped alleviate human suffering resulting from disasters, armed conflict, or other situations of violence. Along with attending to survivors and restoring critical infrastructure, caring for the dead is an essential pillar of humanitarian relief efforts.

Established in 1863, the International Committee of the Red Cross (ICRC) is an impartial, neutral, and independent non-political organization with a large scope of strictly humanitarian activities. Its mandate stems from the Geneva Conventions of 1949, their additional protocols of 1977, and the Statutes of the International Red Cross and Red Crescent Movement.

Since 2003, the ICRC offers forensic assistance exclusively for humanitarian purposes. The International Humanitarian Law (IHL) entails concrete obligations regarding the management of the dead and provides the legal framework for HFA, a concept which the ICRC helped to develop. ICRC's HFA provides technical advice and support to build a sustainable local forensic capacity and medicolegal infrastructure, promoting scientific best practices.

The National Forensic Sciences University (NFSU), established as the Gujarat Forensic Sciences University in 2009, is a unique and first-of-its-kind university offering academic programs, research, and consultancy in specialized areas of forensic sciences and has been elevated to the status of Central University and an Institution of National Importance in 2020. Starting with four courses in three institutes, the university currently has 12 schools and eight centers.

Exploring a feasible and meaningful way to connect operations and academia, NFSU and ICRC joined efforts and established the International Centre for Humanitarian Forensics (ICHF) in 2018 with the vision of being a one-stop Centre of Excellence in Humanitarian Forensics. This partnership between NFSU as a unique higher education institution specializing in forensic sciences and ICRC as a reference organization in HFA is a noteworthy opportunity to address a range of critical policy issues on emerging humanitarian challenges and to promote HFA globally. Acknowledging its domestic relevance, regional footprint, and worldwide potential, the Centre aims to set the standards in HFA focusing on advocacy and leadership, consultancy, research and innovation, and knowledge exchange. In a region prone to disasters, both natural and human induced, with limited and unevenly distributed resources for disaster response, the ICHF leads the way in developing regional and global contingency planning initiatives through transcontinental partnerships fostering important synergy. The Centre offers competitive academic programs producing a well-trained, competent workforce for the global marketplace and tailored institutional training for their professional development.

This novel concept of institutionalizing HFA within an existing university system intends to amplify available multidisciplinary expertise and existing technology and represents the future of humanitarian work to bridge the gap between theory, policy, and implementation while responding to mass fatalities and other relevant areas of HFA.

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### Humanitarian Forensic Action, Higher Education, Caring for the Dead

## E27 Tapping an Overlooked Resource: Preparing and Utilizing Crime Scene Investigators to Recognize and Document Criminal Asphyxiation Cases

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**Learning Overview:** After attending this presentation, attendees will understand the critical need of Crime Scene Investigators (CSI) to identify, document, and preserve potential evidence related to asphyxiation crimes.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the unique challenges presented by asphyxiation crimes and proposing novel solutions, tools, and protocols to utilize CSIs to identify and collect often-missed evidence.

Asphyxiation, whether criminal, accidental, or suicidal, is a process that can be fatal or non-fatal. Asphyxiation directly impedes respiration or blood flow to the brain through various methods, such as strangulation, drowning, and inert gas.<sup>1-3</sup> Criminal asphyxiation occurs in domestic violence, child/elder abuse, sex crimes, state and non-state torture, and human trafficking.<sup>4-7</sup> The diagnosis of fatal asphyxia, criminal or benign, requires an informed and accurate scene investigation and a careful exclusion of underlying injury or disease processes.<sup>8</sup>

Despite the adoption of widespread legislation specifically targeting asphyxiation violence, evidence collection is sparse and convictions are rare.<sup>9</sup> This is partly because criminal asphyxiation often presents with non-specific findings and minimal or no obvious external injury, can be a diagnosis of exclusion, and lends itself to crime scene staging.<sup>10,11</sup> Therefore, these cases require the preservation and collection of often circumstantial evidence present at the initial scene. Capturing this evidence is especially critical when victims and witnesses may be unable to participate beyond the initial scene for fear of retaliation and safety. As such, CSIs are in a unique position to provide a variety of solutions.

It is the job of CSIs to document and process a scene (location, victim, suspect) in its totality as it appears upon arrival. In criminal asphyxiation cases, CSIs are the most apt to capture often-missed evidence, including fingernail scrapings and injuries present on suspects. CSIs may also be the only practitioners who can forensically document initial and follow-up injury presentation. For example, in non-sexual asphyxiation cases, victims are unlikely to receive forensic nurse examinations. CSIs' interactions with victims and suspects can also provide unique opportunities to identify and document signs and symptoms of asphyxia and indicators of abuse. For equivocal fatal cases, CSIs can provide the information necessary for investigators to detect staged scenes and for medical examiners to accurately determine cause and manner of death.

This novel use of CSIs can be achieved by developing protocols, using investigative forms, and implementing training. Protocols are needed to dispatch CSIs to possible criminal asphyxiation scenes and to provide guidance for evidence recognition, documentation, and processing. CSIs must also be properly equipped with knowledge and evidence-based forms in order to effectively work these often-challenging cases. A current obstacle, as will be demonstrated in this presentation, is insufficient CSI training on criminal asphyxiation in the United States as shown by a lack of its mention in CSI textbooks and training syllabi. Overcoming this requires a forensic community response. As a first step in this response, this presentation will introduce tools for CSIs, including investigative forms and protocols for possible asphyxiation scenes. This presentation illustrates the challenges of investigating criminal asphyxiation, offers solutions that CSIs can provide, and introduces CSI tools for investigating strangulation cases. With standardized agency protocols, tools, and specialized criminal asphyxiation training, CSIs can help identify, document, and preserve critical evidence in these cases.

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### Asphyxiation, Strangulation, CSI

**E28 Forensics in the Wilderness: Lessons Learned**

*David Kintz, BS\*, Park County Coroner's Office, Fairplay, CO*

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**Learning Overview:** This synopsis of complicated death scene investigations in the Rocky Mountains from 2010 to 2021 will educate attendees on the pitfalls they may face when conducting a death investigation in the wilderness or other complicated outside situations. There are very few published references on this topic and the best current learning tool we have is sharing real-life scene experience to educate forensic investigators.

**Impact Statement:** The impact of this presentation will be to educate investigators on the unique challenges encountered during wilderness investigations. Most death investigation training is based indoors and has many differences that investigators and other forensics scientists may be unfamiliar with.

This presentation will focus on the complex nature of handling death scene investigations in wilderness settings. These scenes can present difficult circumstances such as extended distance from road travel, weather and scavenger damage to evidence, and life hazards to investigators. When approaching these types of investigations, the investigator must have first, second, and third plans in place and be prepared to innovate at any given notice. Walking in blind to any of these scenes could lead to a compromised investigation or even the loss of life of a responder.

To illustrate the importance in approaching these scenes, this presentation will offer examples with different complications and different manners of death. One example is regarding skeletal remains found 4.5 miles from a trailhead while gaining 2,000ft in elevation. The weather windows were sporadic and presented risk of rain, high wind, and lightening. The investigator took a team of 11 people consisting of coroner staff, anthropologists, human remains detection canines, and Search and Rescue members. The find was reported in the evening. The weather forecast gave the next day as being the clearest and the following days having a higher risk for extreme weather. The team was organized in a rapid manner to be launched at 6:00 a.m. the next day. The hike took 4.5 hours. Upon arrival at the scene, it was found to be a large scattered skeletal scene. With the extent of the hike, time became critical to a successful investigation. The investigator had to break up the team into smaller teams to photograph, Global Positioning System (GPS), describe, and collect all evidence. At the same time, evidence needed to be assessed in the field to answer questions. This action is necessary because returning to the scene is not an easy task in difficult terrain.

A second example will be a found decomposing body that took a 12-hour hike to reach. In this case, the decedent was found to be in a cave situation from which the team was unable to extract. This is a difficult lesson to learn in the field and the purpose will be to drive home to attendees the point of having multiple plans and a team made up of numerous specialties to make the investigation successful.

Throughout the presentation there will be prompts for attendees to assess the situation and decide what they would do with their current knowledge. This will emphasize the point of this presentation to have numerous plans and specialties in place. Many times there will be only one attempt at a successful investigation, and it is imperative that lessons be learned before investigators confront these cases.

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**Wilderness, Death, investigation**

**E29 Homicide Victims and Location, Location, Location: Utilizing Body Dump Patterns and Forensic Archaeology in Outdoor Searches**

*Sharon Moses, PhD, Northern Arizona University, Flagstaff, AZ*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding about relationship and environmental factors that influence homicide offenders' choices about where they dispose of their victims' bodies in outdoor settings. In addition, forensic archaeologists are usually sought after the body disposal site has already been located and the body recovery is complex. Attendees will see that including a forensic archaeologist in the search phase can be beneficial and that, historically, forensic archaeologists have played that role before. Traditional archaeological skill sets include an understanding of environmental factors in order to reconstruct human and animal behaviors at prehistoric and historic sites. Forensic archaeologists bring that skill set to contemporary crime scenes, which includes an understanding of scavenger behaviors and determining a point of origin, if possible, of the body disposal site.

**Impact Statement:** This presentation will impact the forensic community by demonstrating and promoting the value of utilizing forensic archaeologists not only in the body recovery itself, but in determining a search strategy for locating missing or suspected homicide victims. Enhanced search strategies can help law enforcement in locating human remains with more focus of resources. It can also aid in determining a point of origin (where the body was originally left) for surface body dumps depending upon the length of the postmortem interval, environment, taphonomic processes, and scavenging activity.

This presentation will demonstrate that the search as well as the body recovery effort should include forensic archaeologists and anthropologists. It will be informative in that forensic archaeologists have played an important role in search strategies in the past and it is hoped that this presentation will help to restore that interest. The taphonomic processes upon human remains have been the subject of many research efforts over the years, but this presentation will attempt to put those processes in the context of body dispersal by scavengers and forensic archaeologists' understanding of those animal behaviors for ways to narrow the search field and, if possible, calculate a point of origin after the scattered remains are found.

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**Scavengers, Body Dumps, Forensic Archaeology**

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### **E30 How the COVID-19 Pandemic and Related Restrictions Impacted Crime in Italy**

*Francesco Sessa, MS, PhD\*, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; Massimiliano Esposito, MD, Department of Medical, Surgical and Advanced Technologies G.F. Ingrassia, University of Catania, Catania, Italy; Monica Salerno, MD, PhD, Department of Medical, Surgical and Advanced Technologies G.F. Ingrassia, University of Catania, Catania, Italy; Cristoforo Pomara, MD, PhD, Department of Medical, Surgical and Advanced Technologies G.F. Ingrassia, University of Catania, Catania, Italy*

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**Learning Overview:** After attending this presentation, attendees will be able to comprehend how the COVID-19 pandemic and related restrictions have influenced crime activities in Italy.

**Impact Statement:** This presentation will impact the forensic community by providing up-to-date data on crime diffusion in order to present how the forensic investigation has been influenced by the pandemic situation. The data produced will be useful for both forensic practitioners and governments to implement targeted actions in the field of crime prevention.

The outbreak of the COVID-19 emergency worldwide and the subsequent measures taken by each government radically changed the nature of social interactions and economic activities. Many countries have applied a wide range of restrictions, adapting and readjusting their response according to the course of the pandemic. These changes have affected all aspects of life, including criminal activities and forensic investigation.

This research study aims to analyze the impact of the COVID-19 outbreak on the forensic investigation in Italy in the pandemic period. In particular, data were obtained by analyzing national data and those of the Institute of Forensic Medicine of the University of Catania in two periods: before COVID-19 (from February 2019 to January 2020) and during the COVID-19 outbreak (February 2020 to January 2021).

During the lockdowns, several important changes in lifestyles occurred, worsening socio-economic conditions. Analyzing the Italian data, the number of voluntary homicides was reduced (271 cases in 2020, compared to 315 in 2019); the same trend has been confirmed locally (one homicide in 2020 compared to seven in 2019). There was a slight increase in the number of female victims (from 111 in 2019 to 112 in 2020) and in the number of homicides in family relationships (from 94 to 98), maybe due to the weeks of forced cohabitation. It is important to note that in comparing the two analyzed periods, the number of suicides doubled in Italy; analyzing the same data locally, using the data of the Institute of Forensic Medicine of the University of Catania, the situation is dramatically increased: only one suicide is recorded in the pre-COVID period, compared to seven cases in the COVID period. This worrying situation could be related to the measures taken by governments to contain the pandemic, which in Italy included several lockdowns, generating social isolation and economic problems. Moreover, at the national level, the number of femicide-suicides increased among those who killed their wife or partner (from 10 to 21, with a suicide rate of up to 50%), as well as “couple femicides” (from three to five cases). These data are in agreement with other countries that have adopted similar choices in containing the pandemic. In fact, forced domestic cohabitation inevitably influenced the number of domestic violence incidents during the pandemic period. Finally, it is important to highlight that the number of medicolegal disputes has constantly increased due to COVID-19 deaths in both hospitals and nursing homes.

National data and the statistics of the Institute of Legal Medicine of the University of Catania demonstrate how some crimes are changing during this pandemic. According to the discussed data, the COVID-19 outbreak has undoubtedly led to a reduction in the number of voluntary crimes. On the other hand, the number of other types of crime (i.e., domestic violence and/or femicide) has increased considerably. In addition, the number of suicides is certainly alarming: COVID-19 can cause neurological disorders and the pandemic situation has increased mental health problems, such as depression and anxiety. In light of these new issues, governments should inevitably pay attention to them, setting up support programs for citizens. Similarly, the forensic investigator must be aware that in the typology of crimes, the relative investigations are inevitably modified.

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#### **Forensic Sciences, COVID-19, Crime Scene Investigation (CSI)**

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### E31 COVID-19 Impacts on Medical Examiner/Coroner Offices and Their Response to the Pandemic

*BeLinda Weimer, MA\**, RTI International, Research Triangle Park, NC; *Peyton Attaway, BS*, RTI International, Research Triangle Park, NC; *Crystal Daye, MPA*, RTI International, Research Triangle Park, NC; *Camille Gourdet, JD*, RTI International, Research Triangle Park, NC; *Paul Speaker, PhD*, West Virginia University, Morgantown, WV; *Jeri Roper-Miller, PhD\**, RTI International, Research Triangle Park, NC

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**Learning Overview:** After attending this presentation, attendees will understand how COVID-19 has impacted Medical Examiner and Coroner (ME/C) offices' outsourcing practices, staff, and caseloads, and the ways ME/Cs have adapted policies and procedures to address the impacts of COVID-19.

**Impact Statement:** This presentation will provide additional insight into the impact COVID-19 has had on ME/C outsourcing practices and the ways in which ME/Cs have adapted to address these impacts with their already stretched capacity and resources. This presentation will impact the forensics community by bringing awareness to a seldom-researched field and topic.

ME/Cs are a critical component of our nation's criminal justice system. The already existing challenges of high caseloads and dwindling funding, staffing, and resources are now compounded with the COVID-19 pandemic. Because of the COVID-19 pandemic, ME/Cs have had to implement changes in how they operate, especially related to how autopsies are conducted and how bodies are handled.<sup>1</sup> The increase in caseload among ME/Cs due to the COVID-19 pandemic is further exacerbated by the shortage of forensic pathologists and the inability to fill these positions.<sup>2</sup>

To examine the outsourcing practices and how the COVID-19 pandemic has impacted the operation and capacity of ME/Cs, the National Institute of Justice's Forensic Technology Center of Excellence (Award 2016-MU-DX-110) partnered with Project FORESIGHT to conduct a case study with ME/Cs across the country. Focus groups and qualitative interviews were conducted with nine ME/Cs of varying size, location, and type. Findings from the study related to the impact of COVID-19 on ME/C operations and outsourcing and the ways in which ME/Cs have adapted and adjusted procedures to accommodate the additional demands of COVID-19 will be presented.

This study supports local evidence that ME/Cs are doing more with less as their caseloads increase substantially in comparison to their operational capacity. Given the critical role of ME/Cs, research is needed to understand the COVID-19 impact ,not just on ME/C operations, but also on the individual ME/C staff.

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#### COVID-19, Outsourcing, Medical Examiners and Coroners

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## E32 An Examination of Medical Examiner/Coroner (ME/C) Outsourcing Practices

*Peyton Attaway, BS\*, RTI International, Research Triangle Park, NC; BeLinda Weimer, MA, RTI International, Research Triangle Park, NC; Crystal Daye, MPA, RTI International, Research Triangle Park, NC; Camille Gourdet, JD, RTI International, Research Triangle Park, NC; Paul Speaker, PhD, West Virginia University, Morgantown, WV; Jeri Roper-Miller, PhD, RTI International, Research Triangle Park, NC*

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**Learning Overview:** After attending this presentation, attendees will understand the current landscape of ME/C outsourcing practices and how these practices have been adapted to address the opioid epidemic, rise in drug overdoses, and COVID-19.

**Impact Statement:** This presentation will provide attendees with a review of current field practices for the outsourcing of specific ME/C testing functions and the context for which those practices are implemented. This presentation will impact the forensic community by bringing awareness to a seldom-researched field and topic.

The nation's ME/Cs are facing daunting caseloads and depleted funding, staffing, and resources, due to growing threats such as the opioid and other emerging drug crisis and the current COVID-19 pandemic. In addition to these considerations, there has been a shortage of forensic pathologists in the workforce since 2004 and the unprecedented levels of drug-related and other traumatic deaths (e.g., suicides and deaths resulting from motor vehicle incidents) have further exacerbated the impacts of this shortage on the ME/C field.<sup>1</sup> In order to extend their resources, many ME/Cs have turned to outsourcing certain tasks, especially toxicology.

To better understand the strain put on ME/Cs and their current outsourcing practices, the National Institute of Justice's Forensic Technology Center of Excellence (Award 2016-MU-BX-110) partnered with Project FORESIGHT to conduct a study with ME/Cs across the country. Focus groups and qualitative interviews were conducted with nine ME/Cs of varying size, location, and type. The findings related to the current state of ME/C operations and outsourcing and the ways in which ME/Cs have adapted and adjusted procedures to accommodate the additional demands of the opioid epidemic and the current COVID-19 pandemic will be presented.

This study supports anecdotal evidence that the ME/C field is accommodating increases in deaths requiring investigation and a national public health crisis, with little-to-no increase in operational capacity, staffing, health and wellbeing resources, infrastructure, and other assistance. Given the critical role of ME/Cs in the nation's public health and criminal justice fields, research is needed to understand the impact of increasing caseloads with no increase in resources, not just on ME/C operations, but also on the individual ME/C staff.

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- <sup>1</sup> Association of State and Territorial Health Officials. *Brief: Exploring critical issues in the state and territorial public health* (March 2019). <https://astho.org/ASTHOBriefs/Forensic-Pathologist-Shortages/>.

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### Medical Examiners, Coroners, Outsourcing

### E33 “Cutting” for Profit in a Pandemic

Abby Chapman\*, University of Alabama-Birmingham, Birmingham, AL; Cindy Moran, Arkansas State Crime Lab, Little Rock, AR; Daniel Dye, MD, University of Alabama-Birmingham, Birmingham, AL; Brandi McCleskey, MD, University of Alabama-Birmingham, Birmingham, AL

**Learning Overview:** After attending this presentation, attendees will understand the impact that the COVID-19 pandemic has had on the illicit drug supply and deaths associated with fentanyl-laced drugs.

**Impact Statement:** This presentation will impact the forensic science community by illustrating how the opioid epidemic has worsened with the COVID-19 pandemic.

Illicit drug use has had a longstanding history in the United States and has grown to epidemic proportions over the past two decades.<sup>1</sup> Illicit drugs tend to become more popular during times of war or economic downturns. For example, during the Civil War, opiates such as morphine were used as anesthetics and pain killers by doctors whose patients desperately needed amputations.<sup>2,3</sup> Due to this endorsement, morphine began to be prescribed often as a treatment for other maladies and thus began a chain of morphine addictions.<sup>4</sup> Alcohol, once prohibited by the United States government, became a popular source of comfort to those struggling economically during the Great Depression.<sup>5</sup> During the 1980s, a decade that began with a recession, crack cocaine became popular due to its affordable prices in comparison to powder cocaine.<sup>6</sup> This illustrates the drive that drug manufacturers have to increase profits without decreasing the addictive and/or active effects of the drugs. Fentanyl, a synthetic opioid, has been growing in popularity as a “cutting agent” in the North American heroin supply for years.<sup>7</sup> Fentanyl “building blocks” can be obtained easily from the internet for a low cost in wholesale quantities, thus making this drug extremely profitable for manufacturers/dealers.<sup>8</sup>

During the COVID-19 pandemic (beginning in December 2019 with restrictions becoming more stringent in early 2020), fentanyl toxicity-related deaths have increased dramatically across the country.<sup>9</sup> Data from the Jefferson County Coroner/Medical Examiner’s Office (JCCMEO) in Birmingham, AL, demonstrated a 25.6% rise in overall drug-related deaths from 2019 to 2020 and a 100% increase in fentanyl-related deaths during the same time period. The percentage of drug toxicity deaths with fentanyl detected has increased from 40.6% (2019) to 64.6% (2020) to 78.4% (1<sup>st</sup> quarter 2021) to 81.3% (2<sup>nd</sup> quarter 2021). The Arkansas State Crime Lab in Little Rock, AR, experienced an increase of 22.8% overall in drug-related deaths with an 88% increase in those with fentanyl detected. Fentanyl as a component of drugs detected in cases in Arkansas increased from 34.7% (2019) to 53.2% (2020).

These dramatic and disproportionate increases in fentanyl-related deaths are likely due to the ease in which one can acquire it from the internet and from trafficking channels between Mexico and the United States that are still being utilized despite COVID-19 restrictions and border closures. Throughout the pandemic, heroin prices have increased and the availability of fentanyl has decreased; however, purchasing heroin that has been cut with fentanyl will decrease the overall price as well as increase the potency of the drug.<sup>10</sup> In addition to heroin, cocaine and methamphetamine preparations mixed with fentanyl have become the norm rather than the exception during the pandemic. The Centers for Disease Control and Prevention (CDC) report that fentanyl and other synthetic opioids “are the primary driver of the increases in [illicit drug] overdose deaths in the United States, increasing 38.4% during the 12-month period ending May 2020.”<sup>11</sup>

With the latest information from JCCMEO, the continued increases observed in the 1<sup>st</sup> and 2<sup>nd</sup> quarter of 2021 project an even larger number of deaths related to drugs and fentanyl as the pandemic and the epidemic continue to rage on. The issue of fentanyl-related drug deaths has been exacerbated by the COVID-19 pandemic due to economic stressors and supply chain breaks, making mixed drugs the most cost-efficient route, albeit the most dangerous, for both the drug dealer and the drug purchaser. This deadly combination will continue to wreak havoc on the medicolegal death investigation system.

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#### COVID-19, Fentanyl, Opioids

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### E34 The Assessment of Skin Lesions in Trafficking Victims: A Medicolegal Approach

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of a structured interview in a medicolegal examination to correctly discriminate the origin of skin lesions in trafficking victims.

**Impact Statement:** This presentation will impact the forensic science community by underlying the need of a multidisciplinary approach based on an accurate forensic examination along with empathic communication with the patient. The case of a young trafficking victim from west sub-Saharan Africa with peculiar skin lesions will be presented.

According to the United Nations Convention against Transnational Organized Crime, adopted by General Assembly resolution 55/25 of November 15, 2000, trafficking in human beings represents a serious violation of human rights. In recent years, in Italy, there has been a progressive increase in the number of asylum seekers and refugees with a history of exploitation (sexual slavery, forced labor, illegal activities, migrant smuggling). The identification of victims is a necessary prerequisite for assistance and protection measures provided by international laws.<sup>1</sup>

It is important to recognize those who have experienced a trafficking event as early as possible in order to prevent forms of exploitation within the national territory. The Italian procedure for victims of trafficking primarily provides the execution of a structured interview for the purpose of assertion and identification. During the medicolegal anamnestic investigation, thanks to the help of sociological and psychological experts and the use of a “non-judgmental listening” approach, it was possible to create an empathic relationship with the victim. The next step involves carrying out a forensic examination focused on distinctive anthropomorphic and anthropometric traits, as well as particular characteristics, such as scars or injuries. Victims of human trafficking may have skin lesions due to torture or other forms of mistreatment or abuse.<sup>2</sup> They may also present scars related to ethnical practices, such as ritual scarifications.

A self-proclaimed underage woman from west sub-Saharan Africa was forced to travel to Italy by migrant smugglers. She was then mistreated and forced to sell her body until she managed to escape from her persecutors. Since she had no identity documents, she was taken to an Italian hospital in order to assess her age. Therefore, she was visited by a pediatrician and a forensic expert for ascertaining and identifying purposes. The inspection of the left abdominal flank revealed the presence of three parallel oblique scars: the most proximal was 12 x 1.5cm, the central one was 12 x 1cm, and the distal one was 10 x 1.5cm. All the scars appeared diastased and superficial. During the structured interview, thanks to an empathic approach, it was possible to attribute the origin of the scars to traditional medical practices.

As a cultural practice among African tribes, scarification consists in creating wounds in order to cause indelible markings associated with a spiritual, political, or sexual significance.<sup>3</sup> They are generally performed methodically and may show macroscopic differences from other forms of abuse and mistreatment. However, there is a wide range of morphological patterns of lesions perpetrated for different purposes.<sup>4</sup> Scarification marks may even change through the years, becoming hypertrophic or keloidal and thus complicating the differential diagnosis.<sup>5</sup>

In a trafficking victim case, a multidisciplinary approach appears mandatory to correctly distinguish between cultural practices and signs of torture or maltreatment: the medicolegal assessment should be always supported by an empathic communication with the patient, in order to help the victim to reveal sensitive issues from his/her past. Understanding and interpreting such stories gives forensic experts the opportunity to better assess the origin of the lesions.

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#### Human Trafficking, Multidisciplinary Age Estimation, Scarification

## **E35 The Role of MicroRNAs (miRNAs) as New Molecular Biomarkers for Dating the Age of Wound Production: A Systematic Review**

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**Learning Overview:** After attending this presentation, attendees will have a better comprehension of the multiple tools to date the age of wounds in forensic diagnostics.

**Impact Statement:** This presentation will impact the forensic science community by providing a systematic review on the use of immunohistochemical and biochemical techniques to estimate the age of wounds. Moreover, this presentation aims to suggest a new study hypothesis on the role of miRNAs as new molecular biomarkers for dating the age of wound production.

Determination of wound vitality in perimortem injuries and the interpretation of the chronological sequence of events is an important part of forensic work. Antemortem wounds show tissue reaction and consist essentially of acute inflammation. Then, the inflammatory process involves many biochemical molecules (such as miRNAs) and cells (such as neutrophil and mononuclear cell).<sup>1</sup>

Many parameters have been studied to assess the vitality or timing of a skin lesion, including histological (presence of erythrocytes outside the vessels in tissue samples) and immunohistochemical (expression of various inflammatory molecules at different times, such as Selectin E, Selectin P, Cathepsin A and D, fibrin, IL-1 $\beta$ , and others) factors.<sup>2</sup> Nevertheless, this challenge remains open for the forensic community: the identification of the exact moment when the lesion occurs could be very useful in several forensic cases. This literature review aims to analyze the state-of-the-art regarding the estimation of the wound's age, focusing on the use of miRNAs.

MiRNAs are small, non-coding RNA molecules (containing approximately 22 nucleotides) that target genes in a sequence-specific manner to modulate gene expression, mainly by degradation of mRNA or repression of expression.<sup>3</sup> MiRNAs are stable and resistant to degradation. These characteristics make miRNAs potential biomarkers in the forensic practice of identifying antemortem and postmortem wounds, and also for dating lesions.<sup>4</sup> MiRNA are extensively involved in the development of the skin, including epidermal cell proliferation, differentiation, aging, and hair follicle development<sup>7</sup>. MicroRNAs perform special functions in maintaining living cells after death. Therefore, miRNAs continue to be expressed after death, so they can provide important information.<sup>1</sup>

Some databases, from 2000 to June 2019, were searched: MEDLINE<sup>®</sup>, Cochrane Central, Scopus<sup>®</sup>, ScienceDirect<sup>®</sup>, and Google<sup>®</sup> Scholar, using the following keywords: “miRNA,” “wound healing,” “wound age postmortem.” The main keywords were individually searched in association with each of the others.

Several miRNAs were investigated; miRNA-205 and miRNA-21 in particular were often studied. MiRNA-205 has a role in both normal development and cancer and is also highly expressed in healthy skin.<sup>1-6</sup>

MiRNA-21 plays a crucial role in many biological functions and diseases, including development, cancer, cardiovascular diseases, and inflammation. Its production is induced by injured skin and promotes wound healing.<sup>5</sup>

In some studies, the expression of these miRNAs is higher 24 hours after death. Normally, after death, degradation of the cells and their components begins. Similarly, the expression of genes also decreases constantly. The miRNAs studied, on the other hand, are expressed continuously, even 48 hours after death, although at lower concentrations<sup>1</sup>. However, the pattern of degradation of miRNAs after death has not been demonstrated. So this review would like to propose the study of postmortem decremting of miRNAs so their concentration can be correlated to the estimation of wound production time.

In conclusion, the identification of new tools for wound age estimation represents an important challenge for the scientific community. There are few studies in molecular biology. In the past few years, several studies highlighted the role of miRNAs as a highly accurate diagnostic tool. It is important to accurately date the time of production of a wound because it is useful information in the forensic pathologic practice.

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**Estimating Wound Age, MiRNA, Postmortem Investigation**

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**E36 The Dirty Truth: Does Bathing After Sexual Assault Prevent the Development of Combined DNA Index System (CODIS) -Eligible DNA Profiles?**

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**Learning Overview:** Attendees will have a better understanding of the importance of all victims getting tested after sexual assault, regardless of circumstantial factors that may have once prevented them from doing so.

**Impact Statement:** This presentation will impact the forensic science community by presenting the association between developing CODIS-eligible DNA profiles with bathing and time elapsed between assault and examination.

Victims of sexual assault are helped in the criminal justice system by the collection of physical evidence to help identify and convict assailants. DNA is collected from body swabs and reported through sexual assault kits that can then be used to attempt the creation of a CODIS profile. Due to financial or logistic limitations, not every kit is processed and not every swab is tested—sometimes this information is collected and stored for years or even thrown away.<sup>1</sup> For example, cases in which the victim bathed between the assault and the exam are sometimes not tested due to the belief that none of these cases will result in the development of a CODIS-eligible DNA profile.<sup>2</sup> Past studies have shown that it is untrue that bathing removes all traces of DNA.<sup>3</sup>

This study examined bathing in relation to time elapsed between assault and exam. It was seen that bathing was strongly associated with time elapsed, with a greater percentage of victims bathing with longer stretches of time between assault and examination. While there was a decrease in eligibility rates for victims who had bathed prior to their exam, some cases still developed profiles after swabs were taken after longer periods of time (up to eight days) and bathing.

The goal of this study is to encourage victims to get tested after sexual assault, regardless of factors that may have originally discouraged them to do so. In the future, these results could impact which cases are submitted for DNA testing, leading to a higher likelihood of victims receiving justice after suffering sexual assault.

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**CODIS-Eligible STR DNA Profile, Sexual Assault Medical Forensic Examination, Sexual Assault Kits**

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### E37 Increased Sexual Assault Kit Submissions May Not Mean Increased Sexual Assault Prosecutions

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**Learning Overview:** After this presentation, attendees will have increased knowledge about the current relationship between Sexual Assault Kit (SAK) submission rates and sexual assault prosecution rates.

**Impact Statement:** This presentation will impact the forensic science community by providing information comparing increases in SAK submission rates with stagnant sexual assault case processing.

With the discovery of thousands of untested SAKs throughout the United States, research on submission rates of SAKs was conducted in many areas of the United States.<sup>1</sup> The findings of hundreds of thousands of unsubmitted SAKs led to new legislation and policies to submit previously untested SAKs to crime laboratories for analysis. In addition, many jurisdictions also enacted legislation and policies to submit and test all SAKs to avoid future backlogged SAKs. Substantial funding and resources have supported this change to submit and test all SAKs.

The impact of submitting and testing all SAKs on sexual assault prosecution case outcomes should now be evaluated. The purpose of this presentation is to explore the question, “Do increased SAKs submission rates lead to increased sexual assault case prosecutions?”

This study implemented the National Institute of Justice Sexual Assault Nurse Examiner Toolkit on Criminal Case Outcomes in two counties in a United States Mountain-West state to evaluate criminal case outcomes in adult sexual assault cases in which victims had fully collected SAKs and chose to interview with law enforcement for case prosecution.<sup>2</sup> The Toolkit study findings on sexual assault case prosecution outcomes were then compared with SAK submission rates during the same time period (2010–2017).

Between 7% to 10% of sexual assault cases in which victims had fully collected SAKs and requested criminal justice system involvement to investigate and prosecute their cases were successfully prosecuted (plea bargain or trial with conviction). These prosecution percentages stayed fairly constant from 2010 to 2017. Yet, during this same time period, submission and testing rates of SAKs increased from 32% to 89% prior to state legislation mandating the submission and testing of all SAKs.

Resources and funding have been directed toward submission and testing of SAKs. Law enforcement agencies have significantly increased SAK submissions to the state crime laboratory, prior to state legislation mandating submission. Yet, case prosecution rates have remained very low. A shift to increasing resources to criminal justice systems is now recommended to decrease sexual violence and improve justice in adult sexual assault cases. Improving sexual assault case prosecution rates remains an elusive goal, even with increased testing of SAK evidence.<sup>3</sup>

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#### Sexual Assault Kits, Sexual Assault Case Prosecutions, Sexual Assault Kit Legislation

## E38 The Detection and Determination of the Levels of Physiologically Active Substances in Non-Alcoholic Beverages

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**Learning Overview:** The main purpose of this work was to detect the variation in the amount of the ingredients present in popular drinks and how it is a prevalent public health issue.

**Impact Statement:** In the present study, a variety of different parameters were considered, including caffeine, artificial sweeteners such as aspartame, acesulfame potassium, and saccharin, insecticides, and pesticides as well. This study was conducted with the objective of analyzing the quality, safety, and efficacy of the beverages available on the market. This study also shows how some products are mislabeled and misbranded when it comes to the allowed limit of such additives.

Non-alcoholic drinks such as energy drinks, ginger ale, and tonic water drinks have gained immense popularity over time. The most common ingredients present in these drinks are caffeine and artificial sweeteners. This experimental study was aimed at inspecting and determining the levels of ingredients present in these drinks.<sup>1,2</sup> As these drinks claimed to be safe, they were also highly prone to adulteration and mislabeling.

Eight samples of these drinks were collected randomly, all belonging to different brands. They were subjected to chromatographic analysis; the High-Performance Liquid Chromatography (HPLC) method and the Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method were utilized with Ultraviolet (UV) as a detector to determine the content levels of sugar, caffeine, and artificial sweetener, such as aspartame, acesulfame potassium, and saccharin.<sup>3</sup> These samples were evaluated and the results were observed and noted consequently. The calibration standard (2.5mg/L, 5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) was prepared by diluting the above solution or already prepared stock solution with same diluent. Then 20µL of the calibration standard was injected in HPLC. However, out of eight samples, four samples failed the analysis as they contained the components over the limit of quantification according to the label information. The allowed detection limit of caffeine and aspartame in beverages is 25mg/kg. Levels of caffeine were found to be over the detection limit in samples 1,2,5, and 6 and the level of acesulfame K was found to be over the detection limit in sample 2.

Qualitative analysis was conducted to identify the presence of sugar in the beverages. Sugar was reported absent in only one out of the eight samples. Caffeine has been reported to cause toxicity and, forensically, there are special populations that must be perceived as being at a greater hazard risk.<sup>4</sup> Energy drink ingestion could be a potential wellbeing hazard for the general populace, due to the high caffeine dosages. Aspartame metabolites have been reported to cause adverse side effects, including headache, memory-related problems, mood issues, and depression.<sup>5,6</sup>

A variety of different parameters were considered, including caffeine, artificial sweeteners such as aspartame, acesulfame potassium, and saccharin, insecticides, and pesticides.<sup>7,8</sup> This study was performed to analyze the quality, safety, and efficacy of market-available beverages and shows how some products are mislabeled and misbranded regarding the allowed limit of such additives.<sup>9</sup> Previously, some drinks were under controversy as their ingredients were causing an opposing effect on the body, and have failed to conform to the standards. In the marketplace, most energy liquids available have been loaded with sugar, caffeine, and extra calories. The dangers related to their uncontrolled usage had to be highlighted.<sup>10-12</sup>

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**Food Forensics, Caffeine Toxicity, Energy Drinks**

### E39 Improving the Signal Detection Limit of a Portable Raman Spectrometer for Surface Enhanced Raman Spectroscopy (SERS) Detection of Gunshot Residue (GSR)

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**Learning Overview:** Presentation attendees can expect to learn: (1) the usefulness of SERS on a portable spectrometer for the screening of Ethyl Centralite (EC) and Sphenylamine (DPA) in smokeless powders recovered from GSR (or Improvised Explosive Devices [IEDs]); (2) how the analytical figures of merit for EC and DPA such as Signal Detection Limit (SDL), Limit Of Detection (LOD), accuracy, precision, and linearity are affected by the presence of Nanoparticles (NPs); (3) data analysis using Origin<sup>®</sup> software for normalizing, baseline correcting, and comparing spectra; (4) advantages and disadvantages of incorporating portable SERS with Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDX) for the confirmation of both the organic and inorganic components; (5) the type, size, and shape of NP that shows the most enhancement of the DPA and EC signals; and (6) recovery of DPA, EC, and NPs from the screening solution.

**Impact Statement:** This presentation on the effect of SERS on improving the detection limit of organic components of GSR will impact how the forensic science community approaches firearms, and possibly IED-related evidence at crime scenes. The goal is to inform experts about the usefulness of a portable Raman spectrometer as an alternative means for screening GSR in the field to eliminate unnecessary evidence collection followed by costly and time-consuming analysis with SEM/EDX in case that the screening test is negative. Firearm purchases have soared during 2020, implying that there may be an increase in upcoming gun-related homicides with data showing higher rates of gun-related homicides in states with higher levels of firearm ownership.<sup>1,2</sup> Thus, there is a resulting need for more rapid analysis of evidence indicating a gun was fired.

Incorporating this SERS analysis will allow laboratories to focus more on evidence that is presumptively positive, resulting in faster turnaround times for presumptive positive samples and shorter times for prosecuting cases. Faster prosecution leads to faster apprehension of a criminal before they can carry out another crime. Since many laboratories have already stopped carrying out GSR testing, the proposed prescreening will help lighten the load on the laboratories carrying out the confirmatory analysis, and they will subsequently be able to absorb the casework from laboratories that no longer test for GSR.

The portability and simplicity of the Raman spectrometer ensures that rapid screening can be carried out, with minimal training, by law enforcement. Also, Raman analysis is cheaper and simpler than SEM/EDS. This study proposes that this test is more accurate than a color test since it will define an instrument signal and analyte LOD that can be presented in a court of law to make interpretation and presentation of the results less subjective.<sup>3,4</sup> This study also aims to lay the groundwork for future research on the use of a portable microscope Raman for single particle analysis to measure both the organic and inorganic compounds and show its potential as a viable option for GSR confirmation. Finally, this presentation will provide experts with an idea of how sensitive these portable instruments are and the practicality of using them at crime scenes.

The current confirmatory technique for GSR, SEM-EDX is costly and time consuming.<sup>1</sup> In addition, it does not account for changing propellant formulations. While a variety of different techniques have been studied for the analysis of inorganic and organic components, many are still costly and/or time-consuming.<sup>2-6</sup> Raman spectroscopy offers a viable alternative because it is inexpensive, user-friendly, requires minimal sample preparation, has short run times, and can be portable for use at crime scenes. In addition, it can couple with a microscope for single particle analysis and detect both the organic and inorganic components. Advances of Raman analysis of GSR have been summarized, and the usefulness of the portable Raman in analyzing forensic samples has been noted.<sup>7-12</sup> Major drawbacks of Raman spectroscopy, particularly portable Raman spectroscopy, are its low sensitivity and lack of chemical libraries. SERS overcomes low sensitivity by using metal NPs to enhance the analyte's signal via chemical or electromagnetic enhancement.<sup>13,14</sup> This is particularly useful for the detection of forensically relevant trace evidence.<sup>15</sup> The current study examined the effect that two different NPs, iron-cobalt and iron-cobalt with gold, have on the LOD of DPA and EC on a Progeny<sup>®</sup> X2 Rigaku portable Raman spectrometer. While López- López et al. illustrated the usefulness of measuring organic compounds in smokeless powders using a gold substrate and Raman microscopy, these authors carried out their work on thin films, whereas the current work is carried out in solution.<sup>16</sup> Solution analysis allows for minimal preparation time and eliminates the issue of non-homogeneity that would exist in solid samples. In addition, the surface of NPs can more easily be modified with ligands that selectively interact with analytes, thereby increasing the local analyte concentration, which helps avoid pyrolysis and ignition of gunpowder that may occur on films by dissipating the laser energy. Peak height, Full Width at Half Maximum (FWHM), peak area, and peak position were calculated for solutions of DPA and EC in a 1:100 (v/v) ODCB:Hexanes solution ( $n=7$ ). These same analytical figures of merit were then calculated for the same solutions to which iron-cobalt NPs ( $n=3$ ) were added. Excel<sup>®</sup> and OriginPro<sup>®</sup> 2021 software were used to process the data. The calibration curves for peak area and peak intensity versus concentration (up to 300mM) gave R<sup>2</sup> values greater than 0.99. The SDL, Limit Of Quantitation (LOQ), and LOD were lowest for DPA at 1,604 wavenumbers (cm<sup>-1</sup>) and EC at 1,003cm<sup>-1</sup>. The addition of 1 $\mu$ L of iron-cobalt NPs to the DPA and EC solutions resulted in statistically significant changes in peak intensity, peak area, and FWHM for at least one of the DPA and EC peaks. The statistical significance was calculated using a p-test with a significance level of 0.05. This same data will also be reported for solutions containing DPA and EC in the presence of iron-cobalt with gold NPs. A library of the DPA and EC peaks obtained with this portable Raman spectrometer and their relevance for GSR analysis will be reported, in addition to the effect that the NPs have on each peak. Finally, the percent recovery of DPA, EC, and the NPs will be reported so the scientific community understands how the integrity of the probative sample is maintained as well as the potential of this low-cost analysis due to the reusability of the NPs.



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**Gunshot Residue, Nanoparticles, Raman**

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**E40 A New Method for the Preservation of Forensic Fragrance/Odor Evidence for Future Analysis using Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Overview:** Attendees will gain a greater understanding of the issues associated with the collection of fragrances/odors on forensic evidence as well as knowledge about a method to preserve this information for future analysis.

**Impact Statement:** This presentation will discuss the development of a low-cost method to aid in the collection and preservation of volatile trace evidence without disrupting existing DNA evidence.

When fabric/clothing is collected from a crime scene, it is stored in paper evidence bags. This storage method is not airtight, which allows moisture to dissipate and prevents molding. Mold on a sample can degrade DNA evidence, limiting its effectiveness and compromising evidence.<sup>1</sup> Even if an analyst was to detect a fragrance/odor on a piece of evidence, without an effective method to preserve that scent, storage in a paper bag would lead to the eventual dissipation of volatile components. Because DNA is so individualizing, maintaining its integrity will take priority over capturing volatile trace evidence. While DNA is often the most important piece of evidence collected at a crime scene, analysis of other chemical constituents of/on the sample could aid in the investigation.

The goal of this research is to demonstrate a non-destructive method that will allow investigators to collect and preserve fragrance/odor trace evidence without compromising the integrity of any DNA present on the sample. By placing an activated charcoal strip inside the paper evidence bag, investigators can preserve the evidence for future analysis. Activated charcoal strips are typically used in arson investigations for the collection and preservation of volatile components.<sup>2</sup> These strips have been validated and are conventionally used when headspace analysis is not available.

In this study, common fragrances that are readily available in drug or grocery stores were pipetted onto fabric samples of different types (cotton and polyester) with methyl nonanoate as an internal standard. Samples were then placed in both paper bags and in paint cans as a reference for airtight conditions, analogous to arson investigations. An activated charcoal strip was included with each sample for the collection of the fragrance trace evidence. Samples were stored at room temperature for 48 hours and for a month before the strips were removed and placed in airtight vials and stored in the freezer for future analysis, occurring up to nine months later.

Analysis was conducted using Solid-Phase Microextraction (SPME) GC/MS, both directly and with half a mL of ethyl ether as solvent.<sup>3</sup> Results showed that at 48 hours post collection, the fragrances were readily detectable and distinguishable, both in bags and cans. Samples left in bags for one month showed some detectable peaks, but many had dissipated, and the fragrance chromatographic profile was no longer identifiable. Ongoing studies are being conducted to analyze samples after one week, rather than one month, to determine how long fragrances/odors persist under these conditions. Similar to the literature, differences were observed in the retention of odors based on fabric type.<sup>4</sup> It was concluded that a model system for capturing and preserving fragrances/odors on forensic evidence has been developed and is undergoing further evaluation. Forensic evidence collection utilizing this low-cost additional step of adding an activated charcoal strip to preserve scent trace evidence is a promising practice for future investigations.

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**Fragrance/Odor, SPME-GC/MS, Forensic Evidence Preservation**

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**E41 A Chemometrics Study for the Forensic Evidence Classification of Pet Foods Using Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS)**

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**Learning Overview:** Attendees will be familiarized with current chemometrics techniques for the analysis of large chromatographic datasets, applied to the forensic classification of mock forensic evidence in the form of dry pet food.

**Impact Statement:** This study demonstrates that pet food evidence can be classified with SPME-GC/MS data, when combined with multivariate methods such as Principal Component Analysis (PCA) and evaluates the success of classification for both fresh and aged samples.

Pet food and other dry foodstuffs containing protein and grain ingredients have been a source of evidence in previous forensic cases, raising the question of whether such evidence can be classified to its correct brand and make.<sup>1</sup> SPME in combination with GC/MS allows for the detection of several volatile constituents in these non-perishable foodstuffs; however, differences between type and brand of pet food may be difficult to distinguish and quantify based on visual inspection of chromatograms alone.<sup>2</sup> Chemometrics methods, such as PCA, may provide a solution to this issue: large chromatographic datasets can be plotted in two or three dimensions for simple visualization, allowing for assignment of unknowns to a known class.<sup>3</sup>

This study demonstrates the potential of PCA for identification of the brand and type of several classes of commercially available pet food. Four types of dog food were used, two of them—denoted as “Puppy” and “Adult,” respectively—sourced from the same manufacturer and containing the same primary protein source (chicken) as their first ingredient. The other two types of dog food were both from different manufacturers and contained different primary protein sources (beef and buffalo, respectively). Ongoing studies are being conducted to add additional replicates, as well as additional classes that are sourced from different manufacturers and have different primary protein sources.

For each run, kibble was aliquoted to an SPME vial and analyzed with GC/MS. Samples were run fresh out of the bag, one week after the bag was opened, and two weeks after the bag was opened. Of over 30 peaks, 16 were selected and identified for further analysis. Of these, some constituents were present in some samples, and some were present in only some of the samples or classes. A 2D PCA plot shows clear clustering between class when samples are assigned to class independent of freshness, with slight overlap between the “Adult” and “Puppy” classes.

Twelve “unknowns” were compared to the training set using a k-Nearest Neighbors (k-NN) classification algorithm, using k=7 and determining the closest points to the unknown with a Euclidean distance measure of the first three principal components. Only one misclassification occurred: “Puppy” taken fresh out of the bag was incorrectly classified to “Adult.” Therefore, classification of an unknown to brand and specific make had a success rate of 91.67%, while classification to manufacturer had a success rate of 100%.

While this combination of statistical methods demonstrates success in classification of samples by manufacturer, other analytical methods may be needed to classify samples temporally as they change after being opened. Additional investigation of whether individual samples could be classified to assess their age was conducted; however, the success rate of determining week of origin for a specific type of dog food ranges from 33% to 67% with a similar k-NN algorithm (k=3). Despite this limitation, this model study establishes that this combination of analytical techniques and statistical processing has promise for forensic classification of dry foodstuffs containing meat and/or grain ingredients.

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**Pet Food/Foodstuffs, SPME-GC/MS, Principal Component Analysis**

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## E42 Magnetic Resonance Imaging (MRI) of the Skin: Research Hypothesis, Objectives, Limitations, and Postmortem Examples

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**Learning Overview:** The aim of this feasibility study was to investigate the potential role of *ex situ* MRI of the skin after formalin fixation in cases of traumatic death.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the ability to study an *ex situ* fixed-skin sample for forensic purposes with a MRI protocol.

Having been previously overlooked and considered with poor clinical interest, MRI is gaining more and more interest for forensic pathology. This deficiency has to first be linked to a lack of conspicuous data about the relationship between postmortem changes and MRI signal meaning, in addition to the lack of MRI machines in forensic units, so MRI scanners have to be shared with clinical imaging units; the lengthy time of acquisition for the examination from several minutes to an hour for MRI (versus seconds to a few minutes for Computed Tomography [CT], as forensic institutes are likely to have CT rather than MRI scanners); and last but not least, the cost.

This is even more true when it comes to skin, considering that even in the clinical setting it is reserved for a few centers of excellence. However, MRI of the skin could be an innovative diagnostic tool available for tissue characterization in forensics. Although it is difficult to draw up a systematic description of the architecture of the subcutaneous tissue and try to achieve high-quality images of this little structure, above all from postmortem samples, the study hypothesis arises as consequence of the great resolution of contrast of the MRI.

The simplest and most widespread method of evaluating skin lesions has always been the “naked eye” observation, which involves the bias of inter-observer variability (such as the squeezing appraisal of hypostasis after thumb pressure). However, this macroscopic method of evaluation does not always allow the characterization of the tissue/injuries, except with subsequent histological, histochemical, and immunohistochemical investigations. The aim of this study is to present the possible use, through literature review, of MRI in *in vivo* skin examination and analysis. Moreover, samples of *ex vivo* skin explanted and placed in formalin solution underwent a basic and rapid MRI protocol, performed with 1.5 T scanner. Two readers completed image analyses after randomization, blinded to cause of death, in order to recognize the simple tissue architecture of the skin. After having adequate imaging protocol of the normal skin, skin samples of wounds collected during autopsy of traumatic deaths (shotgun injuries, blunt lacerations, and penetrating injuries) were also investigated, in order to identify signal alterations useful for the identification of lesions.

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### Postmortem Magnetic Resonance Imaging, Skin Imaging, Skin Injuries

## E43 The Development of a Rapid Drug Detection Method for Insects Using Paper Spray Ionization-Mass Spectrometry (PSI-MS)

Alexandria Plyler, BS\*, Duquesne University, Pittsburgh, PA

**Learning Overview:** Attendees of this presentation can expect to learn about a toxicological method that, per research, contains a novel combination of methods and instrumentation. Forensic entomotoxicology is still a relatively underdeveloped field, making its study in an academic setting important should it ever be implemented in the field.

**Impact Statement:** PSI-MS as an analytical technique offers great advantages to not only forensic toxicology, but to forensic chemistry and trace evidence professions as well. Its ability to detect analytes with minimal sample preparation without sacrificing sensitivity or specificity can make it a valuable tool by which crime labs can combat the evidence backlog.

Insects have the ability to reflect the chemical environment in which they have developed. Thus, necrogenous insects can reflect the chemical environments of the cadavers on which they have fed. In cases where cadavers are skeletonized, burned, or otherwise too badly damaged for tissues to be collected, forensic scientists can potentially utilize insect larvae as an alternate toxicological matrix to detect drugs in cadavers.<sup>1,2</sup> Traditional techniques for drug detection by Gas Chromatography/Mass Spectrometry (GC/MS) and/or Liquid Chromatography/Mass Spectrometry (LC/MS) often involve lengthy sample preparation, use expensive reagents, and are destructive to important evidence. This poses a problem for crime labs who do not possess the time, funding, or resources to perform such techniques.

Analysis of insects using PSI-MS, an analytical method requiring little-to-no sample preparation, could provide a rapid, cost-effective, and non-destructive alternative form of toxicological analysis. PSI-MS has the distinct advantage of being an ambient ionization technique, allowing for the running of insect samples with virtually no sample preparation at STP without sacrificing sensitivity and specificity. In addition, the use of disposable paper triangles during PSI-MS analysis greatly reduces sample carryover between runs.<sup>3</sup>

To validate a PSI-MS method for insects, several different extraction techniques for drug detection in the model insect *T. molitor* were explored. *T. molitor* larvae first fed on a substrate spiked with Phenethylamine (PEA), a surrogate for illicit monoamine drugs such as methamphetamine and MDMA. Various extraction techniques were then explored, including ethanol extraction, methanol extraction, and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction. In addition, a novel “bug-spray” technique that bypassed insect sample preparation entirely was investigated. By employing each extraction method and analyzing the insect samples using PSI-MS with Collision Induced Dissociation (CID), the most time-efficient, cost-effective, and reliable detection method was developed. The successful implementation of PSI-MS in a forensic setting can pose a useful tool for combating the evidence backlog due to its rapid nature. The ability to study the chemical content of an insect’s environment could have significant potential not only in forensic science, but in conservation studies and the environmental sciences as well.

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Mass Spectrometry, Entomotoxicology, Entomology

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## E44 An Examination of Lipstick Stains Via Fourier Transform Infrared (FTIR) Spectroscopy and Chemometric Methods

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**Learning Overview:** Attendees will learn about the analysis of trace evidence like lipstick stains and its analysis if found at the crime scene through the FTIR technique. The analysis of this chemical data is done through chemometric methods and hence the application of such evidence will be shown as the original study.

**Impact Statement:** For the past decade, there has been a surge in the research work related to the use of chemometric in the analysis of chemical data generated through spectroscopic or chromatographic techniques. Trace evidence has seen the application of chemometric methods combined with analytical techniques for characterization and discrimination of samples, which leads to the informative and representative examinations of samples. This presentation will discuss the examination of cosmetic stains (i.e., lipstick stains) recovered from the crime scene. It is suggested that these new techniques and mathematical/statistical methods are having a statistical significance and are hence highly suitable for forensic casework related to cosmetic forensics.

Among all the cosmetic products, lipsticks are the most commonly used cosmetic product by females worldwide. The composition of lipstick includes inorganic and organic constituents.<sup>1,2</sup> In comparison to other cosmetic products, lipstick stains are the most easily transferred in various types of crimes like rape, sexual assault, murder, child abuse, child trafficking, burglary, and homicides. They may also be found on various other substances like cigarette butts, glasses, clothes, tissue paper, skin, etc. The prevalence of lipstick stains reveals physical contact between the victim and the criminal. Therefore, lipstick stains are considered to be a piece of crucial evidence present at the crime scene. In the present work, 25 lipstick samples are characterized and discriminated using Attenuated Total Reflectance (ATR) FTIR spectroscopy coupled with chemometric and statistical methods.<sup>3</sup> This approach is non-destructive, fast, accurate, and provides reproducible results. The FTIR spectra demonstrated that lipstick stains comprised of various aliphatic and aromatic compounds (e.g., propyl ester of hexanoic acid, silicates, etc.) Further, the discrimination power was calculated using three approaches: visual examination, cluster analysis (Hierarchical Cluster Analysis [HCA] and k-means), and factor analysis methods. The multivariate method combined with t-statistics delivered a higher value of discriminating power (i.e., 100%), which is an improvement over the 99.00% discrimination power of the visual comparison method. The validation of the developed method is done on the basis of predicting unknown samples to their respective brands. The effect of heating (600°C and 700°C) on lipstick stains was also studied. This study establishes a method that provides proof of concept discrimination of lipstick samples. This research can be highly beneficial when it comes to the analysis of trace evidence like lipstick stains encountered at the crime scene. The likelihood of developing a database of lipstick samples using this approach for identifying unknown samples makes it a more important and effective method.

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### Lipstick, IR Spectroscopy, Chemometric

## E45 Multiplex Reverse Transcription Polymerase Chain Reaction (RT-PCR) on a Centrifugal Microfluidic Platform for Field-Forward Messenger RNA (mRNA) Body Fluid Profiling

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**Learning Overview:** After attending this presentation, attendees will have been introduced to a novel rotationally driven microfluidic device that integrates rapid amplification via multiplexed RT-PCR, electrophoresis, and fluorescence detection to detect and differentiate forensically relevant body fluids.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a cost-effective, rapid, portable platform for the detection of mRNA targets present in individual body fluids to identify and contextualize potentially probative evidence.

Forensic body fluid identification is a crucial aspect of criminal investigations that informs subsequent DNA analysis.<sup>1</sup> Unfortunately, this process is rarely straightforward as body fluids of interest are often present in small quantities or mixtures. Conventionally, presumptive assays, though rapid and simple, exhibit poor sensitivity and/or specificity; subsequent confirmatory tests involve time-consuming microscopic or immunological analysis that require operation of complex instrumentation by trained analysts.<sup>2</sup> Among recently emerging alternatives, transcriptomics, or the analysis of RNA, is especially appealing given its amenability for integration into the traditional forensic workflow without compromising the DNA available for analysis.<sup>3</sup> However, conventional instrumentation for RNA-based body fluid identification involves massively parallel sequencing or amplification via RT-PCR—time- and labor-intensive techniques that remain largely tethered to centralized laboratories and require highly skilled operators.<sup>4</sup> As such, the forensic community needs an accurate, portable, sample in-answer out platform that integrates multiple steps (e.g., sample extraction, amplification, electrophoretic separation, and detection) for rapid body fluid identification in the field.

Portable microfluidic systems offer numerous advantages over traditional laboratory techniques, including decreased analysis times, required user intervention, reagent and sample volume requirements, and, ultimately, cost. Microfluidic systems are often readily amenable to use by non-technical personnel, possess enclosed formats that minimize the risk of contamination, and may integrate multiple assays that conventionally require individual benchtop instruments. Here is described one such centrifugal microdevice capable of performing automated RT-PCR amplification and downstream electrophoretic separation on a platform for rapid body fluid identification. Specifically, centrifugal microfluidic devices control fluid flow via rotation, eliminating the need for cumbersome external hardware, such as pumps and syringes, and thus, promoting portability and ease of automation.<sup>5</sup>

The reported rotationally driven device represents significant progress toward development of a miniaturized Total Analysis System (uTAS) capable of performing automated, multiplexed RT-PCR and electrophoretic separation with minimal user intervention. The proposed system reduces the overall time-to-result relative to benchtop instrumentation by leveraging the accelerated PCR kinetics that occurs when working at the microscale.<sup>6</sup> Specifically, total reaction scale was reduced and the surface area-to-volume ratio was drastically increased, enabling the 2-hour RT and 3.5-hour PCR steps to be reduced to ten minutes and one hour, respectively. Multiplexed amplification for differentiation of mixed composition samples was demonstrated using previously validated panels for the target body fluids, including saliva, venous blood, menstrual blood, vaginal fluid, and semen.<sup>7</sup> Future work aims to incorporate automated co-extraction of DNA and RNA onto the microfluidic disc where RNA can be used for on-disc analysis and DNA is reserved for off-disc genetic testing. The work described presents significant progress toward a field-forward, sample in-answer out uTAS for a rapid, cost-effective body fluid identification instrument.

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### Body Fluid Identification, Reverse Transcription Polymerase Chain Reaction (RT-PCR), Microfluidics

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## E46 Preliminary Perspectives of Environmental Conditions on Cocaine's Chemical Odorant Methyl Benzoate Via Dynamic Airflow Sampling

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**Learning Overview:** After attending this presentation, attendees will better understand the effects of controlled target odor delivery on the stability of cocaine's primary chemical odorant, methyl benzoate, when delivered into fluctuating environmental conditions.

**Impact Statement:** This presentation will impact the forensic science community by explaining the effects of controlled odorant airflow delivery into altering environmental conditions. The study focused on using cocaine's chemical odorant, methyl benzoate, under constant room conditions while the odor stimuli output was delivered into changing environmental conditions, depicting an evaluation of odor emission implications for canine detection.

Cocaine is one of the most used drugs in the United States, indicating the need for methods to detect cocaine quickly and accurately. One accepted method for narcotics detection is the use of canines, which are the most common bio-detectors for volatile odor signatures. These odor signatures are composed of Volatile Organic Compounds (VOCs) and are generally constituent compounds in the headspace above the narcotic sample. For example, methyl benzoate is widely accepted as the primary odorant VOC in the headspace of cocaine. Currently, there is a dearth of research as to the role of environmental conditions on canine performance; however, a tangential gap in literature lies in the effects of controlled target odor emission in these changing environments. Thus, the goal of this study is to first validate the olfactometer as an odorant delivery system, then to evaluate the stability of the target odorant as it is being delivered into fluctuating temperature/humidity conditions via a dynamic airflow sampling approach.

Pure methyl benzoate diluted to a  $10^{-2}$  concentration in mineral oil was used as the cocaine odorant analog. The odor was extracted and analyzed with Solid Phase Microextraction (SPME) coupled to Gas Chromatography/Mass Spectrometry (GC/MS). The odor was delivered into an environmental chamber in a controlled amount at an odor/air dilution of  $10^{-2}$  via an air dilution olfactometer, in which the odorant sample was kept at a constant condition of 36°C. The targeted conditions inside of the chamber were hot/humid, hot dry, cold humid, and cold dry. Extractions of the odor were performed by injecting the SPME fiber into the end of the olfactometer output line for 60 seconds while the target odorant was being delivered. The results showed that the air dilution olfactometer was able to deliver varying concentrations of methyl benzoate odor and the SPME-GC/MS methodology was able to detect these changes in concentration. Results depicted that the air dilution olfactometer successfully maintained similar methyl benzoate concentrations at the target odor port despite extreme changes in environmental conditions within the chamber. Despite extreme changes in environmental conditions within the chamber, there was no statistically significant impact on the concentration of methyl benzoate delivered to the target odor port.

SPME-GC/MS was determined to be a valid and reliable methodology for extracting methyl benzoate target odor stimuli. Furthermore, the olfactometer instrumentation proved to be a reliable odorant delivery system in which a consistent amount of methyl benzoate odorant was delivered to the output. However, this study did not find a statistically significant variation in the concentrations of methyl benzoate delivered to the olfactometer output line in different environmental conditions. This indicates that when the target odor source is kept at constant conditions, the concentration of odor emitted remains largely unaffected in the altered environment where it is emitted.

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### Cocaine, Odor, Environmental



**E47 Exploring the Impact of Environmental Differences on Fiber Background Estimation in Forensic Investigation**

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**WITHDRAWN**

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**E48 Backlog Reduction by Implementing Shorter Gas Chromatography/Mass Spectrometry (GC/MS) Confirmation of Seized Drugs**

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**Learning Overview:** This presentation will help attendees understand the use of a shorter and robust confirmation method for the most commonly encountered seized materials which, in turn, helps to control backlogs.

**Impact Statement:** The forensic science community will be able to identify the key steps of analysis that can be shortened effectively for the analysis of seized materials. Furthermore, attendees will be able to compare the work efficiency in pre- and post-implementation scenarios.

Forensic testing laboratories usually face the problem of increasing backlogs due to an increased influx of cases of seized drugs. Laboratories use various methods for controlling backlogs, such as efficient division of labor, increased work force, use of rapid analysis techniques, and increased work hours. Strategies for micro- and macro-administration are required to control increased workloads. Being an immediate neighbor to Afghanistan, increased trafficking and seizure of controlled substances is very common in Pakistan. In Pakistan, cannabis and cannabis products (cannabinoids) comprise nearly 92% of total seizures in a year. This study's laboratory identified that delayed analysis is one of the major causes of backlog build up. Cannabinoids are routinely reported based on the chemical spot test and validated GC/MS method having a total runtime of 12 minutes. Therefore, there was a need to develop and validate a new method with reduced runtime for rapid confirmation of cannabinoids.

Agilent® GC-7890A (DB-5MS-UI column 20m x180µm x0.18µm) coupled with MS-5975C was used in this study. A methanolic solution of cannabinoids (01 µL) was injected in GC-inlet (250°C) using split mode (50:1). Oven temperature was programmed initially at 200°C (1min) and then ramped to 50°C/minute, reaching a final temperature of 300°C (2min). Total runtime of the method was five minutes. MS was operated in scan mode ranging from 53-550amu. The method was validated for selectivity, limit of detection, reproducibility, and matrix effect as per United Nations Office on Drugs and Crime (UNODC) guidelines.

Major cannabinoids (THC, CBN and CBD) were selectively detected in 20 drug samples from different sources, including street samples and secondary standards with sufficient resolution and peak width. Limit of Detection (LOD) of the method was 80µg/ml. Even in the presence of adulterants, the method can be effectively used in routine forensic analysis of cannabinoids. No interference was observed with common adulterants and other controlled substances.

Based on this study, it was concluded that this method is sufficiently specific, selective, and reproducible with enough LOD that has reduced case analysis time up to 60% and improved case turnaround time.

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**Backlog, Cannabinoids, GC/MS**

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## E49 Rapid Fentanyl Sensing Via Biochemical Assay

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**Learning Overview:** After attending this presentation, attendees will understand the dangers of fentanyl and be introduced to a new method of fentanyl and opioid detection for use in both laboratory and field contexts.

**Impact Statement:** This presentation provides a novel, rapid method for the detection of the highly toxic opioid fentanyl.

Opioids are a class of compounds resembling opium in addictive properties or physiological effects. This class includes prescription pain relievers, heroin, and synthetic opioids such as fentanyl. In 2019, the United States alone saw nearly 50,000 deaths associated with opioid overdose.<sup>1</sup> Fentanyl and its analogs are of particular concern. While deaths involving heroin overdose have begun to decline, deaths involving fentanyl and other synthetic opioids have increased significantly.<sup>2</sup> The Drug Enforcement Administration (DEA) Field Divisions labeled fentanyl as “highly available” in 2018 and 2019, meaning drug seekers could easily obtain the substance at any time.<sup>2</sup>

Fentanyl is 50-100 times more potent than morphine and accounts for over half of opiate-related deaths.<sup>3</sup> It is most commonly administered to treat pain after surgery, but illicit manufacturers across the United States and Mexico lead to the high availability of trafficked fentanyl and other opioids.<sup>2,3</sup> Fentanyl and its analogs bind to opioid receptors in the blood and brain. Effects include euphoria, drowsiness, confusion, slowed or labored breathing, and loss of consciousness.<sup>3</sup>

With growing concerns around the use and sale of opioids, newer and safer methods of detection are required. At this time, law enforcement lacks an accurate and reliable method to detect the presence of these compounds in the field. Fentanyl is typically found as a white powder, resembling numerous other substances. To combat this growing issue, non-invasive, bioaffinity-based assays have been developed to detect the presence of opioids and other drugs. This simple system employs a biochemical reaction that elicits a color change visible to the naked eye. This assay is reprogrammable with different enzymes to detect different specific compounds.

The goal of this research is to identify and quantify fentanyl and its analogs on site. Fentanyl and other opioids inhibit butyrylcholinesterase activity. A decline in enzyme function is evaluated colorimetrically via Ultraviolet/Visible (UV-Vis) spectroscopy and a documented reporter reaction. This method is rapid, with results in 30 minutes, and sensitive to the analytes.

Preliminary trials have been performed in a laboratory setting; however, this method is deployable for use in the field. The enzymatic reaction can be bound to a portable strip, similar to the Enzyme-Linked Immuno-Sorbent Assay (ELISA) used in a pregnancy test. The colorimetric change can be compared to a set of known standards visually or quantitatively measured by a smart phone application. Similar strides have already been made by this research group with successful tests for the presence of ethanol and THC.<sup>4,5</sup>

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### Drug Detection, Opioids, Field Deployable

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**E50 A Characterization of Metal-Peroxide Explosive Complexes Using Paper Spray Ionization-Tandem Mass Spectrometry (PSI-MS)**

*Kayla Massari\*, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** Attendees will learn about how the analysis of metal-peroxide complexes using PSI-MS allows the presence of these complexes to be detected and quantified in pre- and post-blast explosive residue.

**Impact Statement:** Organic peroxides have become a common agent in Improvised Explosive Devices (IEDs) because they are accessible for purchase and are susceptible to extremely exothermic reactions due to the instability of the peroxide bond.<sup>1</sup> IEDs have been used in acts of terrorism such as the Oklahoma City bombing and the Olympic Park bombing, and the detection of the explosive component is necessary in terms of eventually identifying the perpetrator who had the means and materials to make the IED.<sup>2</sup> Thus, a reliable and rapid detection method for organic peroxides commonly used in IEDs is of interest for the analysis of pre- and post-blast residue in forensic investigations.

The goal of this project is to develop PSI-MS with Collision-Induced Dissociation (CID) for detection, identification, and quantification of peroxide-based explosives. Benzoyl peroxide and dicumyl peroxide were used as model compounds. To complement experimental studies, Density Functional Theory (DFT) was used to determine probable ion structures. Benzoyl peroxide and dicumyl peroxide samples were spiked with lithium, sodium, and potassium alkali metal cations. The peroxides were also pipetted onto silver-impregnated filter paper. These samples were ionized utilizing the THERMO FISHER™LTQ-XL, and the fragmentation patterns of the peroxide-metal complexes were investigated using CID at the MS<sup>2</sup> stage. DFT calculations of ion structures were performed at the B3LYP/6-311+G(d) and B3LYP/MWB28/6-311+G(d) level of theory.

Initial experiments showed that both dicumyl and benzoyl peroxide form abundant complexes with Lithium Ion (Li<sup>+</sup>), Sodium (Na<sup>+</sup>), Potassium Ion (K<sup>+</sup>), and Silver Ion (Ag<sup>+</sup>) by PSI. In general, PSI produced 1:1 complexes between the peroxides and Li<sup>+</sup>, Na<sup>+</sup>, and K<sup>+</sup>. Studies completed with silver cations and both model peroxides showed that PSI will generate Ag<sup>+</sup> complexes with one or two peroxide ligands. Because the silver-impregnated filter paper allowed for the Ag-peroxide complexes to form, it was determined that a swipe sampling method should be tested with the other metal-peroxide complexes. If this method is successful, it would allow a sample to be swiped from a surface using filter paper and analyzed using PSI with minimal sample preparation. Performance characteristics such as sensitivity and limit of detection are being determined and will also be reported.

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2. *National Academies IED Attack Fact Sheet: Improvised Explosive Devices*. <https://www.dhs.gov/publication/ied-attack-fact-sheet> (accessed September 1).

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**Mass Spectrometry, IED, Peroxide-Based Explosives**

## E51 Determining the Method Threshold of Identification Via Gas Chromatography/Mass Spectrometry (GC/MS) of Weathered Gasoline Extracted From Laminate Flooring

Ellen Hondrogiannis, PhD Towson University, Towson, MD; Allyson Wesley\*, Towson University, Towson, MD

**Learning Overview:** Attendees can expect to learn: (1) the mass/charge ( $m/z$ ) of the ions that are most useful for the quantitative identification of gasoline target compounds identified in weathered gasoline extracted from laminate flooring; (2) the ratio of the base peak ion to qualifier ion 1 as well as the ratio of the base peak ion to qualifier ion 2 in gasoline target compounds in neat gasoline; (3) the percent of weathering above which the ion ratios fall outside the proposed threshold of acceptability; (4) the compounds present in laminate flooring that may interfere in the mass spectral identification of gasoline target compounds; (5) data analysis using Excel® on mass spectral data that the analyst already collects using American Society for Testing and Materials (ASTM) 1618; and (6) repeatability and reproducibility of this method.

**Impact Statement:** This presentation on determining the method threshold of identification of weathered gasoline samples extracted from burnt laminate flooring will impact the forensics community by providing the  $m/z$  of the ions that are most useful for the quantitative identification of gasoline target compounds identified in weathered gasoline extracted from laminate flooring. These  $m/z$  are chosen as the base peak (Q) and two quantifier ions ( $q_1$  and  $q_2$ ) whose ratios  $Q/q_1$  and  $Q/q_2$  fall within a defined limit relative to the same ion ratios identified in the same compounds in neat gasoline. The compounds from the matrix will also be presented as well as their impact on the identification of the gasoline target compounds using this method. The community will learn how to collect this data from data they are already collecting as part of their ignitable liquid analysis using ASTM 1618.

While the effect of weathering on overall pattern identification is well documented, there is no explicit way to determine if a sample is too weathered to be identified as an ignitable liquid. This is up to the discretion of the analyst. While several studies have offered data for probability matching, none outline the limit of detection procedures that should be used to validate the studies. The lack of investigation to determine a quantifiable identification limit when samples are heavily weathered has left a gap in experimental design.

Through studying the effects of weathering and matrix interference on the ion ratios of the target compounds, this work illustrates the use of ion ratios to definitively identify the compounds and thus to establish a threshold below which the analyst can state the compounds are not present in heavily weathered samples extracted from burnt laminate flooring. This method may be used to optimize GC/MS instrument analysis, allow for comparison of data between instruments, and eventually allow a minimum threshold of identification to be set and become part of Quality Assurance programs. Additionally, by incorporating ion ratios into the identification process, analysts will be able to go beyond pattern matching and instead use an identification process that relies on quantifiable data, limiting subjectivity.

Identifying ignitable liquids is a key step in analysis for an arson investigation. The identification of an ignitable liquid at an arson scene not only helps to determine the cause of the fire but also if the fire was started purposefully. The most common ignitable liquid found at arson scenes is gasoline due to its low cost and availability.<sup>1</sup>

Currently, the forensic science community utilizes a pattern-matching technique outlined in ASTM E1618 to identify ignitable liquids from an arson scene.<sup>2</sup> This qualitative procedure is subjective and not straightforward, especially in cases where the sample is heavily weathered and/or there is considerable interference from the matrix. The goal of this work is to define a threshold using mass spectral data above which the analyst can definitively identify the presence of a gasoline target compound. The Organization of Scientific Area Committees defines threshold of identification as the minimum concentration of ignitable liquid identifiable from GC/MS data using accepted pattern identification criteria.<sup>3</sup> This research examines the usefulness of base peak to qualifier ion ratios of six compounds for determining the threshold for identification of gasoline.

The ion ratios were established for each compound using neat gasoline. Ratios were then compared to those obtained for neat gasoline and 90% weathered gasoline spiked onto burnt laminate flooring and recovered from the laminate flooring using headspace extraction (ASTM 1412).<sup>4</sup> The preliminary data show that the compounds extracted from laminate flooring do not interfere with the identification of the gasoline target compounds. In addition, all the ion ratios calculated for indane; 1,2,3,5 tetramethylbenzene (TMB); 5-methylindane; and 1-methylnaphthalene in unextracted neat gasoline fell within  $\pm 20\%$  of the same ion ratios previously reported by Hondrogiannis et al.<sup>5</sup> Ion ratios calculated for 1,2,4,5 TMB and 2-methylnaphthalene fell within 20% for their  $Q/q_1$  ratios but their  $Q/q_2$  ratios fell within 35% of the previous studies compounds. In this study, all but one ion ratio calculated for indane; 1,2,4,5 TMB; 1,2,3,5 TMB; 5-methylindane; 1-methylnaphthalene; and 2-methylnaphthalene in 90% weathered gasoline extracted from laminate flooring fell within 20% of the same ion ratios calculated for the same compounds in neat gasoline. The one ion that did not fall within 20% was  $Q/q_1$  for 1,2,4,5 TMB, and it fell within 30% of the same ion ratios calculated for the same compounds in neat gasoline. This method thus provides a means of definitively identifying the gasoline target compounds. By incorporating ion ratios into the identification process, an analyst will be able to go beyond pattern matching, especially where peaks from gasoline-specific compounds are barely discernable, and instead use an identification process that relies on quantifiable data, limiting subjectivity.

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3. NIST. *OSAC research and development needs*. <https://www.nist.gov/topics/organizationscientific-area-committees-forensic-science/osac-research-and-development-needs> (accessed Mar 20, 2021).

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**Ion Ratios, Weathering, Gasoline**

## E52 Assessing the Rate of Loss of Fibers in Primary, Secondary, and Tertiary Transfers

*Samantha Hadley, BS\*, George Mason University, Fairfax, VA; Steven Burmeister, MS, George Mason University, Fairfax, VA; Cary Oien, MS, Federal Bureau of Investigation, Quantico, VA*

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the impacts different environments have on the movement of fibers.

**Impact Statement:** This presentation will impact the forensic science community by adding to the validity of fibers as evidence, and aid crime scene investigators in making informed decisions about fiber collection and its value.

Trace evidence, while not individual, can be critical in forming investigative leads and adding value in other pieces of evidence. The goal of this research was to further understand how fibers move and transfer with different materials and under different conditions. An additional goal of this research was to assess the likelihood of fibers being found on different materials recovered. This was done by performing three different types of transfers (swipe, touch, and twist) with different substrates (cotton, wool, silk, nylon, carpet, and car seat) to assess how these transfers and substrates will interact with each other in primary, secondary, and tertiary transfers. This study also investigated the impact of force (in the form of weight) and how those different types of motion would be impacted by that force.

This study contains a total of 300 trials with each variable set being run ten times to ensure that potential patterns would be able to emerge. This experiment was conducted inside a transfer chamber, constructed out of wood, and covered in plastic sheeting, to ensure that fibers that were transferred into the environment were able to be recovered. Twenty-five fibers were placed on the initial donor material, then the transfer was conducted to the recipient material. After the transfer, the donor, recipient, and environment were searched, and fibers were counted and recorded. The environmental fibers were removed while the recipient fibers were as undisturbed as possible during counting. The recipient was then the donor for the second transfer and the process was repeated for secondary and tertiary transfers. Each material was tested under the three different transfer motions. The weight portion of the study was only done on the cotton and was also tested under the three transfer motions.

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### Fiber, Transfer, Trace

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**E53 A Physical Assessment of Torture: An Analysis on Medicolegal Degrees of Consistency Expressed According to the Istanbul Protocol**

*Lorenzo Franceschetti, MD\*, University of Brescia, Brescia, Italy and University of Milan, Milan, Italy; Francesca Magli, MSc, MA, University of Milan, Milan, Italy; Chiara Nava, MD, University of Milan, Milan, Italy; Lidia Maggioni, MD, University of Milan, Milan, Italy; Andrea Verzeletti, PhD, University of Brescia, Brescia, Italy; Cristina Cattaneo, PhD, University of Milan, Milan, Italy*

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**Learning Overview:** After attending this presentation, attendees will better understand the critical issues involving the medicolegal assessment of asylum-seeker victims of alleged torture.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of the relevance of the Istanbul Protocol and professional experience in cases involving the assessment of alleged victims of torture.

The Istanbul Protocol is an instrument for the investigation and documentation of torture or other inhuman, degrading, and cruel treatment. The medicolegal section inside the Istanbul Protocol delineated the guidelines for the correct management of physical examination and methods of assigning degrees of consistency in cases of torture. In everyday practice, the assessment of the compatibility of physical evidence with alleged torture is very challenging for the clinical forensic practitioner. Most cases exhibit highly heterogeneous lesions in terms of morphology (margins, shape, size, color, texture, etc.) with no specific features. The examiner therefore may be led to rely subjectively on his or her own experience.

The purpose of this work was to understand whether the experience factor (intended as the number of cases evaluated and the years of experience in this field) influences the evaluation and the final judgement and how objective and statistically relevant a conclusion is according to the Istanbul Protocol.

To this end, a survey containing 11 cases of asylum seekers evaluated by the University Institute of Forensic Medicine of Milan was sent to 30 Italian clinical forensic practitioners. The participants were invited to assign a degree of consistency to each case according to the Istanbul Protocol guidelines.

The answers were statistically grouped, and the inter-rater reliability through Fleiss' Kappa coefficient was calculated by dividing and layering the participants according to the number of cases evaluated (less or more than 25, more than 50), the years of experience (less or more than a year, more than five years), and both variables. Then, the overall agreement among observers was calculated and, afterward, the agreement between observers and an assumed standard reference.

The results showed a trend of improvement in terms of uniformity and homogeneity among the evaluation expressed by raters as the number of cases examined and the years of experience increased. The best Fleiss' Kappa coefficient (0.72) was reached by physicians who had more than five years of experience and visited more than 50 asylum seekers. The same category obtained an agreement of 0.83 with the standard reference.

What emerged from the present research is that a mere application of the Istanbul Protocol is not enough to express an appropriate evaluation, although the protocol tends to yield similar results among expert observers. The evaluations acquire significant values when the practitioner gains experience both in terms of years and number of cases. As for other scientific fields, training (and consequent professional experience) represents the backbone for a proper medicolegal assessment in cases of alleged torture.

Therefore, the proper application of the Istanbul Protocol is only the tip of the iceberg, as the study showed that the judgments are uniform among users with similar experience, albeit with the limitations outlined above. In consideration of the extremely delicate scenarios in which the Istanbul Protocol is used, the time is ripe to consider that the introduction of appropriately trained health professionals "expert in migrations and torture" could lower the risks of misinterpretation and make the assessment as reproducible as possible.

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**Istanbul Protocol, Asylum Seekers, Clinical Forensic Medicine**



**E54 Record Depths: 2019 Deep Water Recovery of “Password 33” at 18,600 Feet**

*Katherine Grosso, MA\*, Armed Forces Medical Examiner System, Dover Air Force Base, DE*

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**Learning Overview:** Attendees will learn about the deep ocean salvage of the United States Navy’s “Password 33,” a C-2A Greyhound lost in the Philippine Sea in November 2017. This presentation will provide insight into the planning and execution of the deepest military recovery of aircraft and human remains on record.

**Impact Statement:** The forensic science community understands better than anyone that every case presents unique challenges, and no two are the same. This presentation will share strategies and findings in deep sea human remains recovery and provide helpful insight for application in the varied underwater search and recovery efforts that one may encounter in medicolegal investigation.

On November 22, 2017, “Password 33,” a United States Navy C-2A Greyhound assigned to VRC-30 in Atsugi, Japan, crashed on carrier approach in the Philippine Sea, representing the first operational loss within the C-2 community in 44 years. Of the 11 active-duty crew and passengers on board, only 8 were rescued before the plane submerged. Search and rescue efforts continued for three days, covering nearly 1,000 square miles of ocean before concluding on November 25, 2017, without recovery. In January 2018, the plane was located at a depth of 18,600 feet by the United States Navy Supervisor of Salvage and Diving (SUPSALV), and the discovery subsequently spurred a multiyear, international, and multidisciplinary effort to recover both the aircraft and the souls on board, then the deepest attempt at aircraft salvage on record.

This presentation will discuss the role played by medicolegal investigators from the Armed Forces Medical Examiner System in the planning, and, ultimately, successful execution of the record-setting recovery in May 2019, to include strategies for human remains recovery in a subsea environment using Remotely Operated underwater Vehicle (ROV) technology, the challenges of executing a large-scale recovery operation at sea, and postmortem findings unique to an abyssal depth aquatic environment.

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**Aviation Mishap, Human Remains Recovery, Deep Ocean Salvage**

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**E55 A Dismembered Pedestrian and a Debris Field: A Case Report of a Fatal Hit and Run**

*Stacey Chepren, MFS\*, University of Florida, Gainesville, FL*

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**Learning Overview:** The goal of this presentation is to show the value of paint chip analysis and investigation of video footage from personal surveillance cameras on private residences.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of a thorough investigation despite the presence of what appear to be obvious facts.

The city of Las Vegas, NV, has a high occurrence of vehicle versus pedestrian accidents. This case took place in the early hours of the morning in the northwest area of the Las Vegas valley. A pedestrian was walking in a crosswalk when he was fatally struck by a Ford® F-150 truck with two occupants. The driver did not stop at the scene and continued to his residence a short distance away. An individual riding a bicycle that morning came upon the scene where he saw a large debris field with pieces of a vehicle, an athletic shoe, and broken headphones. Just a short distance further, he found a dismembered body.

Once the local police were alerted to the incident, they began canvassing the neighborhoods surrounding the scene to look for a damaged vehicle. When they located an F-150 with extensive damage to the front driver's side, they made contact with the owner who claimed to have been asleep all night in his residence. The driver stated his truck must have been stolen and brought back.

Through further investigation, admissions were made by the passenger of the vehicle that the two had been drinking at a bar not far from the residence and that they knew they had hit "something" on the way home. The driver and passenger were captured on video footage at the bar consuming various alcoholic drinks, which was also verified by a bar tab and the bartender on shift. Additionally, investigators noticed a surveillance camera on a neighbor's house directly across from the driver's residence. Upon review of the video footage recorded that morning, it was discovered that the two had returned to the residence just a few hours prior. Not only did they return to the residence in the black F-150, they attempted to clean biological material off of the truck, changed their clothes, came back outside, and drove off in another vehicle that was parked at the residence.

The passenger admitted they had taken the other vehicle back to the scene to collect pieces of the truck, then driven out to the open desert and threw the pieces out. At autopsy, small, black chips of paint were collected from the victim's neck. Paint chip analysis showed multiple layers of paint, evidence the truck had been painted since the factory paint job. A match was made with the custom, aftermarket paint on the black F-150 with the front-end damage.

Throughout the entire case, the driver refused to admit he was the one driving. It was the overwhelming circumstantial evidence collected that showed he was driving the vehicle at the time it struck the pedestrian.

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**Hit and Run, Pedestrian Death, Evidence**

**E56 How Hot Is Too Hot? The Interior Temperature in Unattended Vehicles and the Effect on Children Left Inside**

*Laura Lathrop, MS\*, Sequoias Community College District, Visalia, CA*

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**Learning Overview:** After attending this presentation, attendees will have learned more about how the temperature can rise in vehicles and affect children when left in unattended parked vehicles.

**Impact Statement:** This presentation will impact the forensic science community by giving attendees more knowledge as to temperature increase in vehicles left unattended and not running with children in them. This will provide additional information adding to the already existing literature on this topic. This study is meant to assist investigators in determining how hot vehicles can be when the ambient temperature is at varying degrees. Also, this study will help investigators determine the temperature and time it takes for a child left in a vehicle to succumb to a heat illness.

Since 1998, 887 children have died due to being left unattended in vehicles that are not running. This brings into question: how hot does the vehicle get inside to cause these deaths? How does the ambient air temperature affect the temperature inside a vehicle?

This study attempts to answer some of those questions. This study used temperatures from the Central Valley in Visalia, CA, during the months of July and August. This study was limited to those months, which tend to be the hottest time during the year in this region of California. Differing days were tested with differing ambient temperatures on a vehicle locked for several hours. The interior temperature of the vehicle was taken at various intervals to see how the temperature inside the vehicle rose and at what temperature the interior peaks. Different times of the days were tested to obtain different ambient temperatures. This study was limited to one location: Visalia, CA. The summer months range from 90 to over 100 degrees. As previous research has shown, every region will be a little different on ambient temperatures. The make, model, and color of the vehicle will give different temperatures as will the direction the vehicle is facing and the position in which the vehicle is located. This study adds to the information about the dangers of non-running vehicles and children.

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**Vehicles, Children, Unattended**

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**E57 Have Cocaine, Will Travel: A Case Report of Body Packing**

*Anita Hasert, BS\*, Charleston County Coroner's Office, North Charleston, SC*

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**Learning Overview:** After attending this presentation, attendees will be able to apply their understanding of the various thorough medicolegal death investigation practices. Attendees will understand the importance of utilizing their scene investigation skills, the value of communicating with various organizations, interviewing techniques, and the importance of performing a forensic autopsy. Attendees will also be able to describe the elements of a multidisciplinary approach from the beginning to end of an investigation involving the death investigation of a drug mule.

**Impact Statement:** This presentation will impact the forensic science community by contributing to the literature concerning drug absorption by passive diffusion, the use of the consumption of an antidiarrheal agent, and crucial medicolegal death investigation procedures. The procedures are needed to ensure accurate scene investigation, collaboration among the various agencies, and the need to perform a thorough forensic autopsy. Without the forensic autopsy, the drugs would not have been found, field tested and collected by federal law enforcement. This presentation will also provide information concerning the body packing process, the careful collection of the substances found, and the testing involved.

Passive diffusion is when a drug or drugs that have been consumed spread across a cell membrane from a region of high concentration to one that is of low concentration. An example of high concentration is the gastrointestinal fluids, like the case being presented, and an example of a low concentration region is blood. The rate of drug absorption varies based on the drug that is being introduced, its physiochemical properties, and the route of administration.<sup>1</sup> The purpose of ingesting an antidiarrheal agent is to slow down the intestinal movement for the smuggling of the drug. The drug mule being discussed in this presentation consumed loperamide, which is also known to cause cramping, bloating, gas, and pressure.<sup>2</sup>

The decedent was a Jamaican citizen traveling from Jamaica to New York to reportedly visit his daughter for a few weeks. He was traveling alone. It was determined that he had made multiple trips prior to this incident, but it is unknown if this was his first time traveling as a drug mule. According to the flight attendants, prior to becoming unresponsive while in flight, the decedent had been complaining of feeling cold and had been going back and forth to the bathroom. Once the flight was diverted to Charleston, SC, he was pronounced deceased at the airport. On scene, there was nothing found to assist in the determination of what happened to him. There were no medications found, no medical paperwork, and his luggage was checked by multiple people yielding no clues as to what may have transpired. In interviewing the spouse, the decedent consumed alcohol on occasion, cut back on tobacco use, and would not have used drugs due to the profession he was in. It is important to note that it was later determined that another piece of luggage was found upon debarkation in New York that belonged to the decedent. Once that was checked, drugs were found inside. The decedent left behind a wife and two young children. Based on the social history of the decedent, autopsy findings, drug analysis, and toxicology, the cause of death was determined to be cocaine toxicity. The manner of death was classified as an accident.

**Reference(s):**

- <sup>1.</sup> Drug absorption. <https://www.msmanuals.com/professional/clinical-pharmacology/pharmacokinetics/drug-absorption>.
- <sup>2.</sup> Imodium®. <https://www.imodium.com/>.

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**Cocaine, Mule, Autopsy**

## E58 Necrobiome Succession in Muscle Tissue as a Potential Indicator of Postmortem Interval (PMI)

Anna Wacker, MS\*, Northern Michigan University, Marquette, MI; Josh Sharp, PhD, Northern Michigan University, Marquette, MI; Jane Harris, PhD, Northern Michigan University, Marquette, MI

**Learning Overview:** After attending this presentation, attendees will be familiar with bacterial markers, identified through 16S ribosomal RNA (16S rRNA) metagenomic sequencing of bacterial species present in decomposing human muscle tissue, that contribute to a novel approach for PMI estimation and may aid in the identification of unidentified human remains.

**Impact Statement:** This presentation will impact the forensic science community by providing a new way to estimate PMI by tracking successive necrobiome changes in muscle tissue samples from the arm (deltoid) and the thigh (vastus lateralis) throughout the decomposition process. This presentation will also illuminate the importance of the innate microbiome/necrobiome, unique to certain individuals, that could potentially be used to ascertain aspects of a decedent's diet, lifestyle, and medical history.

Human decomposition is a complex and variable process influenced by innumerable factors such as the weather, innate microbiome, extrinsic microbiome, scavenging activity (including insects), and a myriad of other factors.<sup>1</sup> PMI is a measurement of time since death using one or a combination of methods incorporating, among others, the physical condition of the body, biological and chemical classifications of the substrate surrounding the body, and botanical and entomological analysis.<sup>2</sup> Because of the complexity of decomposition processes and the varying methods used to classify these processes, there is a high variability among individual investigators in their estimates of PMI. By tracking sequential changes in the necrobiome of muscle tissue from samples taken from the arm and leg using 16S rRNA sequencing, one can determine a pattern of succession throughout the decomposition process that could be used to form a reliable, reproducible timeline correlating to PMI in conjunction with Accumulated Degree Days (ADDs). While patterns in necrobiome succession are known to reflect PMI, regardless of environment, location, etc., this study also reveals a novel application for necrobiome data.

Results from this study show a general shift from innate microbiome/necrobiome bacterial organisms to more environmentally associated microbes, such as insect, scavenger, soil, and air microbes. Commonly seen innate human microbes found in early decomposition include Pseudomonadaceae and Lactobacillaceae, while commonly seen extrinsic (later-decomposition) microbes included Wohlfahrtiimonadaceae and Clostridiaceae. When combined with ADD information, the general shift from innate to extrinsic microbes suggests a timeline associated with PMI.

Results from this study also suggest that the presence of specific bacterial organisms may reveal clues about the decedent's diet, lifestyle, overall health, illnesses, and, by extension, possibly cause of death. This could be incredibly important for building the medical and social histories of victims, which may aid identification in medicolegal death investigations. Specific bacterial organisms that have known associations with particular dietary and health-related conditions were isolated in the muscle tissue of the donors used for this study. Bacteria known to be associated with the consumption of dairy, meat, and fish, or that have known associations with particularly healthy or particularly unhealthy gut conditions were found in the muscle tissue of different human donors.<sup>3-5</sup> In each case, a review of the donors' self-reported medical histories supported the bacterial findings. Such information may be particularly useful in generating leads when attempting to build a biological profile for unidentified human remains.

### Reference(s):

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### Necrobiome, Postmortem Interval, Decomposition

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## E59 Human Taphonomy Facilities: Considerations for Establishment in the United Kingdom

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**Learning Overview:** After attending this presentation, attendees will better understand why the United Kingdom does not yet have a Human Taphonomy Facility (HTF), and how establishment and body donation is likely to proceed under the current legislation. Active comparison to countries with existing HTFs will also allow attendees to gain an appreciation for the unique challenges faced by the United Kingdom: identifying the ethical, socioeconomic, and environmental considerations needed for carrying out human taphonomic research.

**Impact Statement:** This presentation will impact the forensic science community by highlighting a gap in United Kingdom research and the global effect this has on human taphonomy research. Analysis of differing international criteria for human tissue donation will also highlight the impact on diversity and inclusion as well as what is locally considered ethical.

As HTFs continue to proliferate around the globe, the United Kingdom remains without access to human taphonomic research. This situation has been made more acute by the United Kingdom's recent departure from the European Union. The lack of domestic research within the United Kingdom has negative implications for the education and training of law enforcement and academia.

In this presentation, the United Kingdom laws relevant to the removal, retention, and disposal of human tissue will be explored along with a review of the history of human research to contextualize the current legislation. The 11 HTFs existing outside of the United Kingdom are discussed, highlighting how they were legally allowed to open and how their body donation protocols differ. Further comparison of consent forms emphasizes the diverging international practices, including time restrictions for returning remains, and the United Kingdom not allowing donations by proxy.

Existing body donation programs operating in the United Kingdom were surveyed and the results will be discussed, comparing consent forms and procedures of medical institutes in the four nations of the United Kingdom: England, Scotland, Wales, and Northern Ireland. Attention is paid to cultural and religious differences, including how the lack of recognition of forensic research as beneficial to public health is limiting research within the United Kingdom.

The geographical constrictions of the United Kingdom are covered, given the limited land available in the comparatively small country. Important issues that could impact the environment are examined, ranging from sustainability to specific public health concerns, such as variant Creutzfeldt-Jakob disease potentially spreading due to scavenging. Also discussed will be the impact on the United Kingdom's economy as well as issues related to the funding of such a facility.

The use of non-human analogs for taphonomy research is analyzed, given this is the only form currently sanctioned in the United Kingdom. Some feel that the United Kingdom could continue to utilize data produced elsewhere; however, extrapolating results from overseas research weakens conclusions due to the United Kingdom-specific climate and entomological presence. The ethical use of animals is also questioned, in the face of access to consenting human subjects.

Finally, the strengths and weaknesses of the United Kingdom are reviewed with a focus on how they could allow it to contribute in a meaningful way to research already conducted, including how it has the potential to lead in diversity of human taphonomy research participants, which remains limited elsewhere.

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### Human Taphonomy, Body Donation Programs, United Kingdom

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## E60 The Effect of Necrophagous Entomofauna on Textile Damage During the Summer in Western Australia

Sotirios Ziogos, MFS<sup>c</sup>\*, Murdoch University, Perth, Western Australia, Australia

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the impact and the artifacts produced by the activity of necrophagous entomofauna on stab-cuts and tear damage on different types of fabric during the postmortem period.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of the effect of carrion insects on clothing and by providing a new body of information that will enhance the investigative role of clothing associated with decomposed and skeletonized remains.

Fatal stabbing incidents are the leading cause of homicides in countries with restricted access to firearms, such as Australia.<sup>1</sup> During a stabbing assault, the distinctive characteristics of a sharp implement will deposit specific features.<sup>2</sup> When the decomposition process impedes the physical examination of a stab wound, damage analysis of the clothing may provide information about the weapon or the actions that caused the injury.<sup>3</sup> Studies have suggested that insect activity associated with decomposition can produce artifacts on textiles, modify perimortem textile damage or produce changes to clothing that imitate indicators of sexual homicides.<sup>4-7</sup> However, the extent of such studies is currently limited and have not been conducted in Australia before. The aim of this research was to identify and characterize the effect of carrion insects on textile damage after a decomposition period during summer in Australia.

The effect of insect activity was analyzed on standardized cuts and tears to three different fabrics (100% cotton, 65% polyester-35% cotton, 80% nylon-20% spandex). Ninety stillborn piglets (*Sus scrofa domestica* L.) were wrapped in one type of fabric each. Each clothed piglet was either stabbed by a stabbing apparatus or had its fabric torn. All piglets were placed simultaneously in a decomposition facility alongside controls of each combination of fabric and damage type, including 9 piglets clothed and intact; 6 piglets unclothed and stabbed or intact; 11 piglets enclosed in boxes; 36 field swatches; and 9 laboratory swatches. Over five sampling periods and until complete skeletonization (7, 12, 18, 26, 47 days since placement), 3 piglets of each type were removed, and entomological samples were collected. The fabric of each piglet was removed, photographed, and stored to dry in laboratory conditions before being analyzed. All controls and fabric swatches were collected on the final sampling day.

Data collection was comprehensive of piglet and fabric samples via field assessment, daily photo and video documentation, static camera recording, and direct collection of insect specimens. Analyses performed covered taphonomic aspects (degree of piglet decomposition), entomological (insect species and instar), and textile damage analysis. The collected fabrics were analyzed on different levels of fabric structure using a stereoscope and digital microscope and through photo and video analysis. Fibers of each sample type were collected and examined using optical microscopy and Scanning Electron Microscopy (SEM).

The results of this research show that stab cuts can adopt morphological characteristics over time that may resemble features of tear damage, whereas tear characteristics also tend to fade gradually. Insect interaction with bloodstains resulted in a distortion of the yarns and of the fabric surface after fly feeding. Due to the weakening of the fabric's structure, insect damage and consequent degradation may occur at the same location. The assessment and comparison of fabrics revealed how parameters such as the type of fabric and elasticity can influence insect damage, with natural fabrics being the most vulnerable. Lastly, this research emphasizes how the presence and the type of fabrics and textile damage affect the interaction of carrion insects with the decomposing medium, and consequently, how the rate of decomposition in a natural environment and in confined spaces is affected.

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### Stab Cuts, Tear Damage, Insect Textile Damage

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**E61 Step-Wise Lead-Poisoning of a Sheep: A Case Report Combining Virtopsy and Autopsy in a Ballistic Veterinary Case**

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**Learning Overview:** This presentation demonstrates cooperation between human and veterinary forensics in a ballistic case; the attendee will learn how to reconstruct gunshot wounds by using not only autopsy but also Postmortem Computed Tomography (PMCT).

**Impact Statement:** This case highlights the successful implementation of postmortem radiology in veterinary pathology, as has been shown in forensic pathology in the past.

On a pasture, police found a sheep with multiple gunshot injuries to the head, inflicted by the sheep's owner, lying on the ground without apparent consciousness but breathing and thus still alive. The sheep subsequently was euthanized and the confiscated carcass was taken to the University of Zurich's Veterinary Pathology for further examination. In cooperation with the Institute for Forensic Medicine, University of Zurich, it was subjected to PMCT scanning and autopsy to document the gunshot injuries.

By Swiss law, animal owners are allowed to kill their own animals, but the method chosen must immediately cause loss of perception and awareness, and also cause death at once. Typical methods are pentobarbital poisoning or using a stun gun aimed at the brain stem. In this case, the sheep's owner claimed that he had tried to kill the animal, which apparently had been suffering from cardiac failure, with two gunshots to the head, in compliance with the law. The main question therefore centered around these central tenets of our legislature, whether the head injuries were able to cause immediate loss of consciousness and cause death—being that the animal was not dead when found by police. The sheep underwent PMCT and autopsy, both which were performed in a close cooperation between the Institute for Veterinary Pathology and the Institute for Forensic Medicine.

Four entrance gunshot wounds were located at the top of the head. Furthermore, aided by the radiological findings, a number of wound channels and retained bullet fragments were noted; from that, the number of shots was at least four but could have been up to seven, based on imaging. According to police investigation, the police could not determine how many shots had been fired. Macroscopically, nasal structures were significantly injured and the olfactory brain was grazed, but no immediately vital areas of the brain, such as the brain stem, appeared to be affected. Diffuse axonal injury may still have occurred and caused loss of consciousness.

A shot sequence could not be ascertained, so it cannot be excluded with certainty that the sheep wasn't unconscious after a first shot, but it appears unlikely. If anything, the considerable number of shots did not cause death immediately. The non-fatality of at least four gunshot head injuries was documented in a veterinary case based on radiological and autopsy findings. This case highlights the successful implementation of postmortem radiology in veterinary pathology, as has been shown in forensic pathology in the past.

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**Ballistics, PMCT, Veterinary**



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## E62 Morphology and Behavior: What Can Dog Bite Wound Morphology Tell Us About the Behavior Involved in Fatal Dog Attacks?

James Crosby, MS\*, University of Florida, Gainesville, FL

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**Learning Overview:** This presentation will examine the relationship between physical morphology of dog bite wounds and the related species-specific contributing behaviors in fatal dog attacks on human victims.

**Impact Statement:** This presentation will expand understanding of dog bite-related fatalities and allow analysis leading to better assessment of human responsibility in civil and criminal venues. This presentation will examine the relationship between reported “accidental” cases and criminal homicide and will examine the use of specific dog bite-related evidence to the investigative process of homicides.

Too often in the past, Dog Bite-Related Fatality (DBRF) cases have been regarded as either horrible “accidents” or as “the dog did it—we are done here.” This study has been able to combine behavioral studies, law enforcement experience, and specific advanced veterinary forensic training to gain a new overview of human fatalities caused by dog bite-related incidents.<sup>1,2</sup> Physical responses to scenes across three continents, direct examination of evidence, and hands-on behavior evaluation of nearly 60 involved dog(s) in over 30 cases has opened new horizons in assessing these incidents. Guidelines for the investigation of DBRF cases have been written and the procedures presented to Law Enforcement and Animal Control investigators internationally.<sup>3</sup>

In addition, hundreds of case reports were examined for commonalities and divergences. Based on wound morphology and behavior analysis, categories of attacks and a comparative rating of human responsibility for the actions of the involved dog(s) has been established. Application of this analysis has led to successful criminal and civil prosecution of owners and others. The general categories identified range from true accidents, through defensive and offensive interactions, to the use of dog bite incidents to conceal and confuse the investigation of criminal homicides. Triggering stimuli are examined, and characteristics of individual dogs are examined in the aftermath of the incidents. This work has involved the comparison of autopsy results, necropsy analysis of accused dogs, dental analysis and comparison, and at times the exclusion of accused dogs and the exoneration of wrongly accused individuals.

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**Dog Bite, Fatal Attack, Bitemarks**

## E63 Are We Barking Up the Wrong Tree When Selecting Detection Canine Candidates?

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**Learning Overview:** After attending this presentation, attendees will better understand the characteristics that make different canine breeds more suitable as detection dogs than others; the genetic connection, if any, to better olfactory capabilities; and the surprising finding that some smaller breeds that are not commonly employed in this capacity may actually perform equally well as those that have been traditionally used for this purpose.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness on the shortcomings of our current selection criteria of canine detection dogs that may not be the most suitable based on their olfactory capabilities or genetic makeup, but that most of these dogs are actually selected due to our familiarity with their breeds. Additionally, it will be pointed out that the working relationship between a detection dog and its handler seems to be the most important factor for the canine team's success.

K-9 dogs are a commodity often utilized worldwide by law enforcement, with certain breeds being chosen most frequently, even though little is understood about the differences in their olfactory ability. Is it possible that the familiarity of seeing certain breeds in that role makes us assume that some are superior to others in this capacity? Multiple studies have been carried out in an effort to determine any genetic difference in olfactory ability, but currently there has been no success. There have been some suggestions that the presence or absence of certain single nucleotide polymorphisms within olfactory gene receptors result in either an increase or a reduction in olfactory performance; however, the process of successful scent detection is likely too complex to rely on a single polymorphism.

This study reviews the work of several international teams to determine if dog breed affects success in detection work, and whether we are currently utilizing the most suitable breed of dogs for this purpose. Comparison of these studies suggests that there are inherent differences in scent detection capabilities between breeds; however, a deeper understanding of why this is must be developed through further research. Due to some contrasting results between studies, there appears to be no single distinguishing factor, and breed selection likely comes down to an amalgamation of features. These include ease of training, motivation for reward, and even physical attributes. Breeds that are typically not reward-inclined are difficult to train to perform detection-related tasks, and results across studies have continually suggested that breeds with a long-standing working relationship with humans may in fact remain the best candidates for the job. A recurring theme throughout most research studies is that canines with a long-standing history of working with humans may remain the best candidates because the relationship between a dog and its handler is paramount for success. Surprisingly, it has been proposed that certain toy dog breeds may perform best when given the opportunity, making a more thorough review of olfactory receptor gene polymorphisms between a large variety of canines necessary to determine any genetic differences in that specific ability. Due to the combination of attributes that make for a successful detection canine and after a thorough review of the literature, this study is of the opinion that the current K-9s are likely to continue enjoying their current job security.

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### K-9 Dogs, Olfaction, Detection

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**E64 An Analysis of the Degradation and Contamination of Canine Training Aids Stored in Semi-Permeable Membrane Devices (SPMDs) and Traditional Storage Conditions Via Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Overview:** After attending this presentation, attendees will be informed about the degradation and contamination of canine training aids stored in traditional storage conditions and SPMDs.

**Impact Statement:** The forensic science community will be informed on the use of SPMDs to store canine training aids as well as cross-contamination between canine training aids stored in SPMDs and traditional storage conditions.

The purity and stability of explosives in canine training aids is of great importance because explosive-detecting canines must be frequently trained with explosive materials. These explosive materials must be free of cross-contamination and have minimal degradation to ensure the canines can accurately identify possible threats. Traditional storage conditions for explosive training aids include plastic bags, Velcro® bags, and drawstring bags. These storage conditions may lead to cross-contamination between the various types of explosive compounds.

For this work, SPMDs were used in addition to traditional storage conditions to monitor the contamination and degradation of 14 explosives used as canine training aids. SPMDs allow for the odor of the compounds to be released while also helping to prevent any contaminants from entering the container. Explosives were stored individually inside of either a traditional storage bag or inside of an SPMD. A set of each storage type was stored at Indiana University – Purdue University Indianapolis to act as the control and a set was stored at an Indianapolis Metropolitan Police Department location and were actively utilized as canine training aids. A sample from each storage type at both locations was collected at 0, 3, 6, and 9 months and analyzed using FTIR spectroscopy and GC/MS with solid-phase microextraction. For most explosives analyzed via FTIR, no degradation or contamination was identified as most spectra were visually indistinguishable. Chemical changes were seen in the FTIR spectrum for urea nitrate as the sample became dehydrated over time. Chemical changes were also seen in the FTIR spectrum for the cast booster sample. These changes are likely due to the heterogeneity of the sample and not from degradation or contamination. GC/MS spectra show cross-contamination from ethylene glycol dinitrate and/or 2,3-dimethyl-2,3-dinitrobutane across almost all samples regardless of storage condition.

FTIR and GC/MS analyses show that canine training aid explosives show no signs of degradation when stored in either traditional storage conditions or in a SPMD; however, cross-contamination is still of concern.

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**FTIR, GC/MS, Canine Training Aids**

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## E65 Forensic Nurse Roles: Exploring the Future Within a Modern Forensic Science World

*Joyce Williams, DNP, RN\*, Stevenson University, Owings Mills, MD*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the diversity of forensic nursing roles and how they contribute to clinical and research work.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of forensic nursing clinical skills, research abilities, and contributions to partnerships with governmental and non-governmental agencies.

Forensic nurses most frequently work in hospitals, community anti-violence programs, coroner's and medical examiners offices, correctional institutions, and psychiatric hospitals to meet and surpass the challenges of a modern forensic science world. They may also be called on following mass disasters or community crisis situations. Forensic nurses bring a nursing perspective to an investigation as they play an integral role in bridging the gap between law and medicine. A portion of the work in the clinical setting involves observing, recording, and preserving evidence, and photographing injuries for evidence of wrongdoing that may eventually be used in court. Striving to improve crime victims' health outcomes, their interactions are notably within the legal arena, systems of care, and community agencies as well as with individuals. They interface with law enforcement and legal teams to support victims, often providing fact-based testimony in court cases. As grueling as this work can be, it comes with many benefits, including fighting for victims of abuse.

A brief introduction of the various careers of forensic nurses will be examined and how this specialization impacts the clinical arena, research and development, outside agency collaboration, and risk management will be discussed. While it will be impossible to enumerate all the positions in which forensic nurses excel, several appointments will be discussed.

Professionally, forensic nurses specialize following academic education and licensure plus practice in a medical setting. Most settings require that a nurse practice for a specific length of time before specialization. Depending upon what area of specialty is chosen, the nurse will engage in the required training, clinical hours, and possibly an internship with mentorship.

Some advanced practice forensic nurses choose to conduct research. This opportunity is widely available as is evidenced in the work of Ann Wolbert Burgess, who focused on developing ways to assess and treat trauma in rape victims. The military has engaged forensic nurses to analyze wounding in combat in order to better prevent mortality. Forensic nurses, as injury analysts, research morbidity and mortality injuries in clinical settings to determine intentional from unintentional wounds. The use of Artificial Intelligence (AI) is one emerging specialty focusing on the impact of medical interventions that stray from evidence-based practice causing harm to the patient. AI translates accumulated data into contextually relevant knowledge and conclusions to impact appropriate actions.

Forensic nurses are valuable partners with governmental and non-governmental agencies. They operate as subject matter experts to create legislation to improve criminal legal and community-based responses to domestic violence, sexual assault, dating violence, and more. Under the radar positions are employment with the Federal Bureau of Investigation (FBI) where the Bureau trains forensic nurses to be special agents or work as detectives investigating major crimes utilizing their expertise in an occupation that benefits from their specialized knowledge. The Federal Emergency Management Agency (FEMA) utilizes forensic nurses in several positions as leaders and specialists who deploy to disaster situations. The Organization of Scientific Area Committees (OSAC) makes use of forensic nurses who collaborate as members of a subcommittee to develop national standards for use in areas of practice.

The diverse roles of forensic nurses are wide-spread and their contributions to the medicolegal community affect the lives of many globally. The innovators of forensic nursing science have influenced the future of a profession that is continually developing.

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### Forensic Nurse, Roles, Partnerships

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## E66 A Global Response in the Frontiers of Forensic Nursing Science

Virginia A. Lynch, MSN\*, *Forensic Nurse Consultants, International, Divide, CO*

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**Learning Overview:** Attendees will learn the background of native cultures in countries within the accepted beliefs of culture, tradition, religions, and superstitions with patients, health care practitioners, and law enforcement's acceptance or rejection of the forensic nurse examiner. Attendees will learn the unique obstacles and benefits of introducing the forensic nursing concept where professional respect is not recognized in existing nursing roles by physicians and other barriers to education in international countries.

**Impact Statement:** This presentation will impact the urgency of founding forensic nursing science as a global initiative in health care and human rights that can help reduce and prevent violence against women, children, and vulnerable populations. This presentation will improve attendees knowledge, competence, and performance of trans-cultural nursing in the United States and internationally.

In a post-modern world filled with criminal violence, the forensic nurse has created a previously unrecognized resource for victims of domestic and international terrorism that often starts within the sociocultural, political, and religious traditions of the past.<sup>1</sup> The United States has led the advancement of forensic nursing throughout its 50 states as it continues to expand worldwide throughout developed and developing nations. The discipline of forensic nursing is well established in North America and our physicians are grateful. Some of our most successful programs were influenced by the American Academy of Forensic Sciences (AAFS), the first professional organization to recognize and encourage Forensic Nursing as a scientific discipline and that has now elevated this emergent science to the status of a section of its own: the Forensic Nursing Science Section.<sup>2,3</sup>

The global forensic community is paying attention in 57 countries. The AAFS is renowned worldwide; their influence and leadership speak for its prestigious reputation. In addition to the United States, several countries with emerging initiatives in forensic nursing include those with major issues of prevalent crime, interpersonal and sexual violence, and the need to ensure human rights for women and children where they have been limited by cultural norms. There are multiple driving forces for the development of forensic nursing within these areas in which nurse-run clinics are the only source of health care and nurses certify death.

The first education program for forensic nurses outside of North America was in South Africa. The Northern Cape Institute of Forensic Studies in Nursing has become the precedence for other countries. This original program was initiated by Dr. J.E. Els, Chief of Forensic Medical Services, in a place so small it was difficult to find on a map. As so often happens, this action brought attention to the AAFS, the United Nations, and to the South African Minister of Health, leading the way for it to become the first country to designate forensic nursing as a national priority program in all nine provinces.

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### Terrorism, Nurse-Run Clinics, Emerging Initiatives

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**E67 Responding to the Future Challenges of Emergency Preparedness in a Modern Forensic Science World**

*Joyce Williams, DNP, RN\*, Stevenson University, Owings Mills, MD*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of public health measures in emergency preparedness, how forensic nurses impact all phases of disaster preparedness, and thoughts on how to prepare for the future.

**Impact Statement:** This presentation will demonstrate the failures encountered during the current pandemic and how forensic nurses are valuable assets in all phases of emergency preparedness.

In the past decade, 2.6 billion people globally have been upended by earthquakes, floods, wildfires, and other natural disasters. Now, the global coronavirus pandemic has killed more than four million people worldwide. These crises disproportionately affect people of color, those with low incomes, those experiencing housing insecurity, and those with limited access to health care and transportation, ultimately exacerbating health disparities. For too long, the United States has overinvested in treating illness and underinvested in promoting health and well-being and preventing disease. Even before COVID-19 illuminated disparities and exacerbated inequities in the United States, forensic nurses were advocating for better care and access for individuals, families, and communities.

COVID-19 has shifted the world to a worrisome state of epic proportion. Pandemics occur relatively frequently, surfacing every few decades on average (for example, influenza). The biggest factor affecting the spread is the current interconnectedness of the world. Diseases move rather quickly now. This current pandemic revealed the fragility and brittleness among our public health system and health care delivery, supply chains, and supply chain logistics. The broad impact across not only the United States, but also globally, impacted the economy. While the direct cost of the pandemic exceeds \$16 trillion, the long-term health costs for the treatment of just one affected person is estimated at \$26,000 or more. However, the structural costs to communities are multi-faceted, including education, diversity, income disparity, and more.<sup>1</sup>

During the height of the COVID-19 pandemic in the United States, data-collection shortcomings overwhelmed state health departments, impeding health officials' abilities to identify and contain outbreaks and raised the curtain on long-neglected state and local health systems. A six-month *Politico* investigation found delays in reporting COVID test results, sometimes by weeks, arcane computer programs hindering data collection, and severely understaffed contact tracing programs. Despite these limitations, federal agencies relied on states for case and death counts. Effective contact tracing failed.<sup>2</sup>

Effective response to humanitarian events is a recognized action of disaster teams, including forensic nurses, in efforts to secure and support nations. The response to the pandemic by health care was forceful, yet public health measures were ineffective, limited by poor communication and information sharing across regional and international borders. While there was a high degree of mobilization and adaptability to respond to the current needs of equipment and other public health deliverables, the overall result was reactive, lacking overall preparedness.

What preparations are needed for the future? Innovation must embrace technologies for early detection of threats, interventions, and improvements in nanobiophysics technology for mass scale surveillance. Can we employ smarter quarantine systems and next-generation health care delivery? The last influenza pandemic was in 2009 and recurs every 20–35 years. The G20 Nations Summit urged governments to promote the creation of a global surveillance network to detect the harbingers of a potential new pandemic. However, other threats exist: global warming, the emergence of new diseases, and antibiotic resistance. It is time to create a broader vision, listen to and appraise the data, and collaborate on a whole-of-society approach.

The medical and forensic science professionals are a cadre of valuable individuals who have the expertise to prepare and respond to mass casualty events. Forensic nurses are the frontline as the Chief Nursing Officer Assistant Secretary for Preparedness and Response (ASPR), Senior Advisor to National Disaster Medical System, or on disaster teams providing rapid assessment of the injured, casualty surges, displaced populations, violence prevention, care of human remains, and the mental health of victims, families, or staff. The expertise of forensic nurses as contributors to all aspects of preparedness is important to better design and monitor surveillance and reduce risks to populations.

**Reference(s):**

- <sup>1</sup> <https://www.mckinsey.com/industries/public-and-social-sector/our-insights/how-might-the-covid-19-pandemic-end>.
- <sup>2</sup> <https://www.politico.com/news/2021/08/15/inside-americas-covid-data-gap-502565>.

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**Forensic Nurses, Pandemic, Preparedness**

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## E68 Forensic Nursing Science: A Contemporary Response to Humanitarian and Human Rights

Virginia A. Lynch, MSN\*, Forensic Nurse Consultants, International, Divide, CO

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**Learning Overview:** The forensic nurse represents a previously unrecognized resource for expanding forensic services and is poised to fill a crucial role in the examination of patients, including victims or suspects of child and elderly abuse, intimate partner and sexual violence, domestic homicide, liability issues, persecution, and torture, and violations of human rights. Forensic nurses collaborate with other health care, criminal justice, and forensic sciences professionals. A shortage of clinical forensic physicians and forensic pathologists has alerted a need for additional health care resources worldwide.

**Impact Statement:** The American Academy of Forensic Sciences (AAFS) forensic nurses, other forensic specialists, and attendees will better understand the forensic aspects of nursing and recognize the need for forensically skilled nurses in the investigation of trauma, questioned death, and accountability to the AAFS codes of ethics and legislation of nursing practice.

“The current world situation is such that a nurse may become involved in innumerable circumstances requiring action on her/his part to safeguard human rights.”<sup>1</sup> As the science of nursing merged with the forensic sciences and criminal justice systems, a distinctive discipline has evolved: forensic nursing science. This emergent science has evolved in response to the consequences of global human violence. Problematic social, cultural, and political situations corresponding to the escalation of reported violence reinforces the need to define the dynamics, processes, and guidelines for forensic nursing practice. Forensic nursing is an essential discipline that recognizes human violence through a contemporary domain of scientific knowledge, health care, and human rights.

Forensic nursing science is objective and unbiased. It applies equally to either side of civil and criminal laws or other legal matters. Forensic nurses work with forensic scientist to help determine the truth in a court of law through the proper documentation, recovery of clinical forensic evidence, and testify in court as required. Forensic nursing care helps to provide improved health and justice outcomes.<sup>2</sup>

The need for nursing actions to safeguard human rights is not restricted to times of political upheaval and war. It pertains to the abuse or maltreatment of patients or others, whether witnessed or suspected.<sup>3</sup> Forensic nurses are expected to perform examinations on victims or suspects before they are subjected to forms of interrogation, which may include torture. To ensure that the nurse is prepared and knowledgeable to provide or prevent the predictable treatment involved that meets the standards of International Law (IL) requires competence through a formidable forensic and nursing education including the Istanbul Protocol.

Health and justice organizations supporting forensic nursing principles and philosophies on human rights include the AAFS, Humanitarian and Human Rights Resource Center (HHRRC), International Council of Nursing (ICN), International Committee of the Red Cross (ICRC), International Association of Forensic Nurses (IAFN), and Amnesty International (AI). Each group encourages all members to engage in humanitarian and human rights applications to advance global health and justice.

### Reference(s):

1. Amnesty International. *Ethical Codes and Declarations Relevant to the Health Professions*. 3rd Revised Edition: 1994: 1 Easton Street, London WC1X 8DJ, United Kingdom.
2. Lynch, V.A. Proposal for a new scientific discipline: Forensic nursing. *Proceedings of the American Academy of Forensic Sciences, 43<sup>rd</sup> Annual Scientific Meeting*, Anaheim, California; 1991.
3. International Council of Nurses (ICN). *Position statement: Nurses and human rights*. Geneva: International Council of Nurses; 1983.

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### Unbiased, Truth, Torture

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## E69 Forensic Nurse Hospitalist: The Comprehensive Role of the Forensic Nurse in a Hospital Setting

*Kelly Berishaj, DNP\*, Oakland University, Rochester, MI; Christina Boyland, MSN, Turning Point, Macomb, MI; Kristin Reinink, MSN, Spectrum Health, Grand Rapids, MI; Virginia Lynch, MSN, Forensic Nurse Consultants, International, Divide, CO*

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**Learning Overview:** Attendees will learn about the unique and evolving role of the forensic nurse hospitalist and the impact of this role on patients, providers, and health care systems when trauma is involved. Attendees will learn about the need for such a role in terms of the number of patients seen in the hospital setting who experience trauma, maltreatment, and violence and require expert care from specially educated and trained forensic nurses following best practice recommendations and national guidelines. Finally, educational considerations for nurses interested in pursuing the Forensic Nurse Hospitalist (FNH) role and how the role may be implemented into acute care settings will be described.

**Impact Statement:** This presentation will impact attendees in that they will learn about a new and unique role that may be currently lacking in their health care agency. Implementing the FNH role has the ability to positively impact health and legal outcomes of patients who have experienced trauma. The FNH can also impact and improve health care provider performance when delivering care to patient-victims. Finally, the FNH can help impact health care systems by serving as a leader in the development of policy, procedure, education, and consultation when trauma, violence, or maltreatment is involved.

The need for forensic nurses in the hospital setting continues to grow daily, and now more than ever, is becoming a crucial factor in successful patient outcomes.<sup>1</sup> “Forensic Nurse Hospitalist” is proposed as a new term to describe the role of the nurse who is qualified by licensure and education as a specialist in forensic nursing and practices specifically in the hospital setting. The FNH can provide comprehensive, medicolegal care to patients receiving treatment for trauma-related injury and questionable death in the acute care setting.

This presentation highlights the need for and importance of the FNH and describes the multifaceted responsibilities of the role as a transformational leader in health care today. The FH Conceptual Model was developed to illustrate the three pillars of influence impacted by the FNH role: patients, health care providers, and health care systems. It is vital that health care systems employ FNHs to deliver expert patient care, offer specialized consultation and collaboration, and implement system-wide policy and educational initiatives to best meet the needs of patients who have experienced intentional or unintentional trauma.

### Reference(s):

- <sup>1</sup> Pasqualone, G.A. (2015). The relationship between the forensic nurse in the emergency department and law enforcement officials. *Critical Care Nursing Quarterly*, 38(1), 36-48. 10.1097/CNQ.0000000000000047.

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### Forensic Nursing, Role Development, Specialty Advancement



## E70 Exploring the Conditions, Challenges, and Situations for Nurses Involved in Forced Organ Transplants in China

Nancy Cabelus, DNP\*, University of New Haven, New Haven, CT

**Learning Overview:** This presentation will define forced organ harvesting; identify vulnerable groups targeted by traffickers; and discuss the importance of the forensic nurse's role in preventing forced organ harvesting.

**Impact Statement:** This presentation will create awareness of underground criminal activity involving vulnerable populations and an international health care network.

During the past two decades, awareness of the crime of forced organ harvesting in China has been documented by international experts in ethics, medicine, and law. Organ transplant surgeries have quadrupled in China as compared to countries around the world.<sup>1</sup> The major source of donor organs is from northwest China and the donor population is a Chinese group of Falun Gong practitioners known as Uyghurs. Falun Gong is a spiritual practice based on truthfulness, compassion, and tolerance. Uyghurs are a particularly passive people and are therefore soft targets for predators who sit in powerful political positions and dictate who is next to die.

In the Western world, organ transplantation in the best of circumstances may take months or even years to find a suitable donor-recipient match. However, in China, a donor may be located in just a few days. The donor source is often a Falun Gong practitioner, a prisoner of conscience, who may be executed in exchange for the lucrative sale of viable organs on the black market.

Warranting further investigation is the role of the (forensic) nurse in the process of organ procurement, surgical interventions, and post-operative care of patients. There are limited resources that discuss the participatory role of nurses. China Organ Harvest Research Center reported that 10,000-15,000 transplants per year occur in China.<sup>2</sup> What is unknown is exactly how this underground network of matching donors to international recipients happens and how nurses are recruited to participate in this process. Are nurses forced to participate in this process or otherwise face punishment or death? If so, this certainly could be considered a form of labor trafficking and would pique the interest of law enforcement and human rights groups alike.

Forensic nurses practice where health care and legal systems join with forensic science. Nursing care of organ transplant patients, whether donors or recipients, is within the scope of forensic nursing practice. "The American Nurses Association believes that respect for the inherent dignity, worth, unique attributes, and human rights of all individuals is a fundamental principle."<sup>3</sup> The International Association of Forensic Nurses supports that "when faced with ethical choices, forensic nurses should use recognized ethical frameworks for decision making. The guiding principles of ethical decision making are autonomy, justice, beneficence, and nonmaleficence."<sup>4</sup>

The International Council of Nurses Position Statement on Nurses and Human Rights states that, "Nurses have an obligation to safeguard, respect, and actively promote people's health rights at all times and in all places."<sup>5</sup> Nurses are accountable to obtain informed consent in an understandable language from patients prior to their "consenting to treatment or procedures, including participation in research."<sup>5</sup> "Inherent in nursing is a respect for human rights, cultural rights, the right to life and choice, and dignity, and to be treated with respect."<sup>5</sup> Therefore, nurses who participate in the practice of forced organ harvesting would deliberately violate this protective code of values, ethics, and human rights.

Xie, J.F. et al. assessed the attitude and impact factors toward organ transplantation and donation among nurses in China.<sup>6</sup> Of 536 questionnaires received, most nurses reported negative attitudes toward organ transplantation and donation. Only 33.4% of the nurses stated they would donate their organs after death and only 38.2% were willing to register in the national organ donation system. Since nurses would be an important group in the organ donation screening and transplantation processes, their negative attitude can significantly influence donor registration. Investigating what Chinese nurses know about forced organ harvesting could reveal information critical to stopping organ traffickers internationally and saving innocent lives.

### Reference(s):

1. Matas, D.; Trey, T. (2012). *State Organs: Transplant Abuse in China*. Ontario Canada: Seraphim.
2. Caplan, A. (July 2018). (Foreword.) *Transplant abuse in China continues despite claims of reform*. China Organ Harvest Research Center. <https://www.chinaorganharvest.org/>.
3. American Nurses Association (2016). *The nurse's role in ethics and human rights*. <https://www.nursingworld.org/practice-policy/nursing-excellence/official-position-statements/id/the-nurses-role-in-ethics-and-human-rights/>.
4. International Association of Forensic Nurses.(2020). <https://www.forensicnurses.org/>.
5. International Council of Nurses. (2020). *Code of Ethics for Nurses*. Retrieved from: <https://www.icn.ch/news/international-council-nurses-launches-consultation-revise-code-ethics-nurses>.
6. Xie, J.F.; Wang, C.Y.; He, G.P.; Ming, Y.Z.; Wan, Q.Q.; Liu, J.; Gong, L.N.; Liu, L.F. (July-August 2017). Attitude and Impact factors toward organ transplantation and donation among transplantation nurses in China. *Transplant proceedings* Vol. 49, Issue 6., pp. 1226-1231).

### Forced Organ Harvesting, Forensic Nursing, Human Rights

**E71 The Forensic Nurse Response to Elder Abuse: Meeting and Surpassing the Challenges**

*Amy Carney, PhD\*, California State University San Marcos, San Marcos, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of elder maltreatment and the forensic nursing response to detection, intervention, and resolution.

**Impact Statement:** This presentation will impact the forensic community by broadening the education of those working with victims of violence and enhance the ability to intervene in maltreatment and prosecute the abuser.

The actual study of elder abuse is relatively recent in the United States. Claude Pepper, as the head of the House Select Committee on Aging, conducted hearing in the 1970s on elder mistreatment, then called “granny bashing,” but research and resources were years in coming. Like child abuse and domestic violence, harm to an older relative was considered a family matter and stayed behind closed doors. Much of early theory was based on patterns of family violence that had been studied in child abuse and the battered wife syndrome. With the growing understanding of the nature and extent of elder mistreatment came the awareness of the lack of forensic and medical markers that could be useful in the identification of abuse. There was no research that could assist investigators in establishing lacerations, bruising, fractures, or decubiti as elements of abuse or neglect. The aging patient frequently has physiological characteristics or medical conditions, which can mask or mimic markers of neglect and abuse, making it even more difficult to detect or evaluate. Unless an actual crime could be proven, such as homicide, charges were rarely filed against a suspected abuser. Arrest and prosecution for financial abuse or neglect was unheard of. Testimony by older patients could be difficult to obtain.

Specialization by forensic nurses in the field of elder maltreatment has increased the detection and prosecution of elder abuse. Advanced assessment, understanding of federal, state, and local statutes, and interfacing with law enforcement are some of the challenges forensic nursing confronts when working with abused and neglected elders. This presentation describes the role of the forensic nurse in meeting the needs of the elderly abuse victim.

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**Forensic Nursing, Elder Abuse, Maltreatment**

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**E72 Forensic Nursing Science as an Indispensable Core Service in Modern Patient Care: The First Implementation Outcomes in Switzerland**

*Valeria Kaegi, RN\*, University of Zurich, Zurich, Switzerland; Michael J. Thali, MD, University of Zurich, Zurich, Switzerland; Rosa Maria Martinez, MD, University of Zurich, Zurich, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the developmental milestones of forensic nursing in Switzerland, taking into consideration the differences in the health care system, educational programs for forensic nurses, and practical implementation of forensic nursing.

**Impact Statement:** This presentation will have an impact on modern patient care, especially in cases of interpersonal violence without the filing of criminal charges.

Victims of interpersonal violence are often admitted to hospital emergency departments before they are identified by hospital staff as victims of crime due to a lack of forensic training for nurses and clinical physicians. Therefore, the general demand for forensic nursing is increasingly growing in many Swiss hospitals and institutions, especially in cases arising from interpersonal violence without reported criminal charges. For this reason, the educational offering for nurses in the field of forensic nursing continues to be promoted, advanced, and developed in Switzerland. A sustainable implementation of this nursing specialization requires, among other things, clearly defined roles and competence descriptions for forensic nurses as well as defined institutional structures and frameworks.<sup>1,2</sup> The Swiss Association Forensic Nurses (SAFN) has established the overarching competencies of forensic nurses, which enable the involved interfaces to call upon the resources and skills of forensically educated nurses in a demand-oriented and efficient manner. Efficient forensic case management in a hospital allows for timely documentation that can be used in court and proper handling of potential evidence so that it can be used efficiently if criminal charges are later filed. The implementation of forensic nursing and the superordinate competencies as well as the possible areas of application of forensically qualified nurses in Switzerland is comprehensively illustrated.

**Reference(s):**

- <sup>1.</sup> Lynch, V.A.; Duval Barber, J. (2011). *Forensic Nursing Science*. Elsevier, Mosby, St. Louis, MO.
- <sup>2.</sup> Butt, John. (1993). Forensic Nursing: Diversity in education and practice. Cited in V. Lynch, *J Psychosc Nurs Ment Health Serv*, 31(11), 7-14.

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**Forensic Nursing, Patient Care, Competencies and Limits**

## **E73 Improving Care for the Sexual Assault Patient**

*Kathleen Sekula, PhD\*, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** This presentation describes the importance of increasing the number of Sexual Assault Nurse Examiners (SANEs) in practice in rural and underserved areas throughout the United States; explains the process of supporting SANEs in practice to decrease turnover; and outlines a mentoring program.

**Impact Statement:** This is an area of great need. There are not enough SANEs in practice to cover all areas where SANEs are needed. In addition, there is a high turnover with SANEs. It is challenging work. Grants have afforded us the opportunity to train many more SANEs and to create programs that support retention.

In many areas of the United States, no expert SANEs are available to provide services for survivors of sexual assault. Because of a lack of SANEs, resulting in poor care, survivors often do not report their assaults, with adverse effects on physical and mental health. Duquesne University School of Nursing (DUSON) received funding from the Health Resources and Services Administration (HRSA) to provide the education, clinical experience, and telehealth mentoring for Registered Nurses (RNs), Advanced Practice Registered Nurses (APRNs), and Forensic Nurses (FN) who choose to become certified SANEs. DUSON offers a rigorous SANE online didactic course and has offered a well-established forensic nursing graduate program since 2005. However, students are challenged in securing the necessary follow-up clinical experience because they lack qualified community-based SANE experts who can serve as preceptors.

The goals of the grant were to: (1) establish a coalition of academic and clinical partners, actively educating, training, or employing SANE nurses to leverage their resources to increase the number of certified SANE nurses nationally, with a focus on nurses serving rural and underserved areas; (2) develop and implement a model for comprehensive SANE education and training (didactic, clinical, and telehealth mentorship) with a focus on nurses serving rural and underserved areas; (3) prepare nurses for the national certification exam, including both participants in the proposed program and other nurses seeking SANE certification; and (4) develop a sustainability plan to support future nurses preparing for SANE certification and entering practice.

Outcomes currently include the education of over 170 nurses from 23 states, with 72 newly certified. The team is committed to disseminating outcomes and is confident that this innovative program utilizing telehealth mentoring will provide a model for the future.

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### **SANE—A Practice, Rural/Underserved, Mentoring**

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## E74 The Development and Successful Implementation of a State-Wide Sexual Assault Nurse Examiner (SANE) Coordinator Role: A Rural Perspective

*Matthew Gamette, MS, Idaho State Police Forensic Services, Meridian, ID; Deborah Wetherelt, RN\*, Idaho State Police Forensic Services, Meridian, ID*

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**Learning Overview:** After attending this presentation, attendees will have an understanding of some of the difficulties encountered in improving access to standardized care of the victim who has been sexually assaulted in a rural state and how those difficulties can be successfully surmounted.

**Impact Statement:** This presentation will impact the forensic science community by promoting an understanding of: (1) the importance of creating a state-wide working group (Multidisciplinary Team [MDT]) around sexual assault; (2) having a State Coordinator to facilitate standardized SANE training; (3) the benefits of the unique collaboration between a Nurse Coordinator and the state lab; (4) forensic practitioners partnering with the Nurse Coordinator to improve evidence collection and patient care; and (5) the need for state-wide protocols.

In 2018, the Idaho Legislature created the unfunded role of the Idaho Sexual Assault Nurse Examiner/Sexual Assault Response Team (SANE/SART) Coordinator; after some discussion, it was decided the position would be housed under the direction of the State Crime Lab Director to facilitate close relationships between the forensic scientists and the forensic nurse in the role and grant funding was procured.

The initial goal of the program was to train 250 Registered Nurses (RNs) as SANEs. After a trying hiring process, an experienced forensic nurse was brought on board and immediately began working to establish the educational curriculum that would meet the requirements of the International Association of Forensic Nurses Educational Planning Table for the 40-hour Adult/Adolescent SANE Course.<sup>1</sup> The first Coordinator trained 51 nurses, but moved on after eight months; it was several months before a replacement was found.

The second (and current) Coordinator had no direct forensic experience, but had extensive leadership and project management experience; the initial focus was for the Coordinator to contract with forensic nurses that were certified as Sexual Assault Nurse Examiner-Adult/Adolescents (SANE-As) to teach the 40-hour SANE-A course, schedule multiple courses across the state, create networks of forensic partners across the state, and begin the arduous process of becoming SANE-certified in less than a year.

COVID, of course, hampered the travel and education plans and only 31 nurses were trained in 2020. The Coordinator identified SANE programs across the state and successfully networked them together through group emails, quarterly virtual meetings of the Idaho SANE Program Leaders, quarterly newsletters, and personal contact. As recognition of the program and Coordinator grew, standardized evidence collection improved as forensic nurses reached out to the Coordinator for direction; the Coordinator and forensic scientists collaborated in creating a method to identify discrepancies in evidence collection of the Sexual Assault Evidence Collection Kits (SAECKs) and provide direct feedback to the collector, further improving collection methods and techniques.

The 40-hour course approval expired in 2021 and was revamped; eight courses have been scheduled across Idaho for 2021, and the Coordinator is encouraging organizations to schedule into 2022 to ensure continued education for staff. A four-hour *Medical Forensic Examinations for the ED Staff* was created to ensure emergency room physicians and nurses could properly complete evidence collection when a SANE was not available.

As with all successful programs, there is no time to rest; expansion of the program through collaboration with law enforcement and other partners is being developed as Idaho continues to pursue excellence in forensic practice.

### Reference(s):

1. Lechner, M. MSN, RN, CNS, SANE-A, SANE-P et al. (2018). *Sexual Assault Nurse Examiner (SANE) Education Guidelines*. International Association of Forensic Nurses. [https://cdn.ymaws.com/www.forensicnurses.org/resource/resmgr/education/2018\\_sa](https://cdn.ymaws.com/www.forensicnurses.org/resource/resmgr/education/2018_sa).

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**Idaho, Sexual Assault Evidence Collection Kit, SANE**

**E75 The Standardization of Sexual Assault Examination Kits in the United States: An Exploration of the Role Sexual Assault Nurse Examiners (SANEs) and Telehealth Have on the Sexual Assault Examination Process**

*Amy Southall-Malone, MFS\*, Mississippi Forensics Laboratory, Pearl, MS*

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**WITHDRAWN**

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**E76 The Sexual Assault Nurse Examiner (SANE): A Responsive Strategy to Global Sexual Violence**

*Jamie Ferrell, MBA, BSN, RN\*, Memorial Hermann Healthcare System, Houston, TX*

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the specialized SANEs evidence-based practice across the life span, the improved health impact on survivors of sexual violence, the quality of forensic science results, and responsiveness to the judicial process.

**Impact Statement:** This presentation will impact the forensic science community by providing a blueprint that can be implemented globally as a response to sexual victimization through the evidence-based practice of the specialized adult or pediatric SANE.

Sexual violence and its associated trauma are pervasive through each society. Adverse childhood experiences such as sexual assault have been linked to leading causes of adult morbidity and mortality. Reducing this trauma is critical to avoiding multiple negative health and socioeconomic outcomes in adulthood. The likelihood of restoration following sexual violence is increased for victims who have access to medical professionals skilled in trauma-informed care that has important implications for their future health and well-being.<sup>1</sup>

Experience indicates that successful prosecutions of sexual crimes require the coordination and cooperation of both skilled health care practitioners and the criminal justice system. Strategies to improve evidence-based global standards of care for victims of all ages post-sexual assault necessitate specialized forensic nursing education to include pertinent knowledge of the law. The patients' history of sexual assault and a comprehensive assessment are the important predictors to assist with decisions of evidence recovery.

SANEs are Registered Nurse health care providers qualified in the examination and evaluation of physical trauma with an emphasis on maximizing evidence recovery while minimizing emotional trauma with trauma-informed compassionate care modalities. Education of the SANE specialist in adult and pediatric populations entails a 41-hour adult SANE course and a minimum 120 hours of clinical training, observation, and precepted exams prior to caring for these patients.<sup>2</sup> Pediatric SANE education is an additional 43-hour pediatric SANE course along with a minimum of 104 hours of clinical training, observation, and precepted exams.<sup>2</sup>

Best-specimen recovery has inordinate significance in the criminal investigation of sexual violence. The SANE understands that the application of science and technology continues to enhance the implication of biomedical, trace, and physical evidence to unprecedented levels within the judicial domain. Sources of potential evidence are continually expanding with the advanced sensitivity of DNA testing. SANEs are specifically educated in the understanding of the evolution of DNA with saliva transfer or touch DNA in strangulation.<sup>3</sup>

Access to SANEs facilitates improved evidence collection kit results for sexual assault patients.<sup>4,5</sup>

The SANE serves as a clinical liaison to the criminal investigation and is available to testify in court when required. While clinical responsibilities center on competency for quality patient outcomes, the application of scientific technology involving electronic documentation and digital imaging advances clinical outcomes and operational efficiency.

**Reference(s):**

1. Merrick, M.T.; Ford, D.C.; Ports, K.A., et al. Vital Signs: Estimated Proportion of Adult Health Problems Attributable to Adverse Childhood Experiences and Implications for Prevention — 25 States, 2015–2017. *MMWR Morb Mortal Wkly Rep* 2019;68:999-1005.
2. International Association of Forensic Nurses. *Sexual Assault Nurse Examiner Education (SANE) Guidelines 2018*.
3. National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach. *National Institute of Justice (NIJ)*, 2017.
4. Sievers, V. et al: Sexual assault evidence collection more accurate when completed by sexual assault nurse examiners. *Journal of Emergency Nursing*, Vol 29, Issue 6, 511 – 514.
5. Campbell, Rebecca. 2009. Systems change analysis of SANE programs: Identifying the mediating mechanisms of criminal justice system impact: project summary. Rockville, MD. *National Criminal Justice Reference Service*. <http://purl.fdlp.gov/GPO/gpo27139>.

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**Sexual Assault Nurse Examiner, Sexual Violence, Evidence-Based**

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**E77 Sexual Assault Victim and Assault Characteristics and Development of Combined DNA Index System (CODIS) -Eligible Short Tandem Repeat (STR) DNA Profiles**

*Julie Valentine, PhD\*, Brigham Young University, Provo, UT; Sam Payne, PhD, Brigham Young University, Provo, UT; Leslie Miles, DNP, Brigham Young University, Provo, UT; Luke Johnson, Brigham Young University, Provo, UT; Connor Alder, Brigham Young University, Provo, UT*

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**Learning Overview:** After attending this presentation, attendees will have increased knowledge about sexual assault victim and assault characteristics associated with the development of CODIS-eligible STR DNA profiles from sexual assault evidence kits.

**Impact Statement:** This presentation will impact the forensic science community by adding to the few studies exploring victim and assault variables and association with DNA analysis development of STR DNA CODIS-eligible profiles.

When individuals report sexual assault within ~120 hours of the assault, they have the option of having evidence collected during Sexual Assault Medical Forensic Examinations (SAMFEs).<sup>1</sup> During SAMFEs, forensic examiners collect information about the victim and the assault to guide the examination and evidence collection. The SAMFE examination form is generally given with the Sexual Assault Kit (SAK) to law enforcement professionals who are responsible for submitting SAKs to crime laboratories for analysis.

The purpose of this study was to explore victim and assault variables contained in the SAMFE forms and the subsequent development of STR Deoxyribonucleic Acid (DNA) profiles eligible for entry in the Federal Bureau of Investigation Combined DNA Index System (CODIS). This retrospective, exploratory study of ~5,000 SAKs found that the following variables were statistically significant in increased development of STR DNA CODIS-eligible profiles: age; gender; time between assault and exam; suspect actions of grabbing/holding, physically hitting and/or strangling victim; vaginal-penile penetration; suspect oral contact on victim genitals and other body parts; ejaculation; higher number of penetrative acts; and victim with anogenital injuries and non-anogenital injuries. Variables associated with decreased development of STR DNA CODIS-eligible profiles included the following: increasing length of time between assault and exam; bathing/showering after assault and prior to exam; multiple suspects; and suspected drug-facilitated sexual assault. Logistic regression models were calculated to explore predictive power of statistically significant variables.

Exploring variables associated with development of STR DNA CODIS-eligible profiles is beneficial in practice and policy decisions for Sexual Assault Nurse Examiners (SANEs), forensic examiners, and forensic scientists. With the increased submission and testing of SAKs, crime labs are exploring options to improve workflow of SAK DNA analysis.<sup>2</sup> Other studies have been conducted and found variables associated with development of STR DNA CODIS-eligible profiles include time between assault and exam, history of consensual sexual contact, and bathing/showering.<sup>3</sup>

**Reference(s):**

1. National Institute of Justice. (2017). *National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach*. Retrieved from <https://www.ncjrs.gov/pdffiles1/nij/250384.pdf>
2. Bazinet, M.; Larose, J.; Comte, S.N.J.; Primeau, M.; Lapointe, M.; Paquet, C.; Landry, C.; Croteau, L.; Gingras, F. (2020). Data driven optimization of sexual assault case processing. *Forensic Science International: Synergy*, 2, 162-172.
3. Kerka, J.E.; Heckman, D.J.; Albert, J.H.; Sprague, J.E.; Maddox, L.O. (2018). Statistical modeling on the case information from the Ohio Attorney General's sexual assault kit initiative. *Journal of Forensic Sciences*, 63(4), 1122-1133.

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**Sexual Assault, Sexual Assault Medical Forensic Examinations, STR DNA Codis-Eligible Profiles**



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## 578 An Estimation of Error Rates in Library Matching of Forensic Textile Evidence Classified by Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) Spectroscopy

Lauren Bouju Davies\*, Seattle University, Seattle, WA; Braeden Camarota, Seattle University, Seattle, WA; Kristen Skogerboe, PhD, Seattle University, Seattle, WA

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**Learning Overview:** The goal of this presentation is to instruct attendees on the use of ATR/FTIR in the creation of a spectra database for use in the classification of unknown forensic textile samples. This presentation will evaluate how fabrics are classified using spectral library matching of unknown fabric samples to a fabric library.

**Impact Statement:** This presentation will impact the forensic science community by providing an estimate of the error rate on an ATR/FTIR instrument when used to match textiles to a created in-house fabric library. Spectra from the in-house library were compared to published spectra to ensure accuracy. In forensic science, estimating the accuracy of testing methods is a point of scrutiny, so this project assesses the error rate of ATR/FTIR when applied to classification of forensic textile evidence.<sup>1</sup>

FTIR is a powerful tool for identifying fabric type but is limited by the availability of public domain databases of FTIR spectra.<sup>2</sup> Forensic laboratories and educational institutions must either make their own library or purchase one of the limited fabrics FTIR databases in existence.<sup>3</sup> This study establishes a fabric library using FTIR and evaluates its effectiveness in classification of unknown textile types. Pieces of fabric were tested instead of individual fibers because the ATR allows for the sampling of textile evidence, including very fragile textile samples, without causing further damage.

This project created a digital and accompanying physical fabric library of 36 “pure” samples (100% one fiber type) and 30 mixed samples (at least two fiber types). Both natural and synthetic fibers were cataloged. At least two different samples from different sources for each pure fabric type were tested, including varying colors, weaves, and dye techniques to simulate the diversity of samples that would arise in routine forensic case work.

For this study, a PerkinElmer® Spectrum Two ATR/FTIR was used to collect four scans of each sample to generate one spectrum. A total of 66 different fabrics were scanned to create an in-house fabric library. The same samples were then re-run through the ATR/FTIR as unknowns and searched against the fabric library. The top three matches and accompanying search scores, determined using the software’s built-in Euclidean distance algorithm, were recorded. All samples were tested in replicate. Three separate libraries were designated for testing purposes: (1) pure,  $n=36$ , (2) mixed,  $n=30$ , and (3) combined,  $n=66$ . For estimation of error rate, pure samples were run against a pure library, mixed samples were run against a mixed-only database, mixed samples were run against a pure library, and all samples were run against the combined library.

Generally, pure samples were more accurately matched by the fabric library compared to the mixed samples. To date, all pure samples matched to the same fabric type, but did not always self-match. Some mixed fabrics did not match by fabric type or self-match, indicating that the usefulness of FTIR for identifying unknown mixed fiber samples may be limited. Mixed fibers may need additional methodologies, including multivariate analysis techniques, to accurately classify the fiber types.<sup>4</sup> Challenges specific to mixed samples will be presented. An operator well-trained in FTIR usage and spectra analysis can increase the accuracy of textile identification.

These results provide a baseline of data that show the success of spectral database matching. The results also illuminate problems with spectral matching of fabric types. This shows a path forward to improve the accuracy of textile classification using ATR/FTIR.

### Reference(s):

1. 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.
2. Vahur, S.; Teearu, A.; Peets, P. et al. ATR-FT-IR spectral collection of conservation materials in the extended region of 4000-80  $\text{cm}^{-1}$ . *Analytical Bioanalytical Chemistry* 408, 3373–3379 (2016). <https://doi.org/10.1007/s00216-016-9411-5>.
3. Institute of Chemistry University of Tartu, Estonia. (n.d.). *ATR-FT-IR Spectra of Various Fibres*. Database of ATR-FT-IR spectra of various materials. <https://spectra.chem.ut.ee/textile-fibres/>.
4. Peets, P.; Leito, I.; Pelt, J.; Vahur, S. Identification and classification of textile fibres using ATR-FT-IR spectroscopy with chemometric methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Volume 173, 2017, Pages 175-181.

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### Forensic Fabric/Textiles, Library Matching Error Rate, ATR/FTIR

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**E79 An Assessment of Heat Damage on Fourier Transform Infrared (FTIR) Spectral Characteristics of Various Fabric Samples for Applications to Forensic Clothing Examination**

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**Learning Overview:** After attending this presentation, attendees will have been introduced to the use of Attenuated Total Reflection/Fourier-Transform Infrared (ATR/FTIR) spectroscopy to distinguish between burnt, unburnt, and partially burnt fabric samples. Research results of controlled fabric damage demonstrate differences in functional groups present within samples that are changed or removed due to pyrolysis. This is applicable in cases where forensic fiber evidence was subjected to fire or other types of heat, conditions common in arson or when attempts to conceal a crime are made.

**Impact Statement:** This presentation will impact the forensic science community by assessing the utility of FTIR for the evaluation of heat damage to fabrics, including damage produced at fire crime scenes, by friction, hot gases from firearms, and discarded evidence from human rights cases. This work represents an extensive controlled study that evaluates changes in FTIR spectra when fabric is exposed to heat, and examines whether these would impact conclusions drawn from other testing of the evidence.

In this study, fabrics comprising ten different fiber types (cotton, silk, wool, polyester, rayon, nylon, leather, linen, lyocell, and polypropylene) were subject to heat damage from an open flame and from convection heat, with each type burned independently and paired with each other.<sup>1</sup> Heat damage occurred in visually distinct stages (evaluated microscopically and reported separately), with each damage stage assessed by ATR/FTIR.

There are limited, albeit growing, scientific studies dealing with heat damage of fabrics in a forensic context.<sup>2,3</sup> For example, light chars and burns can be difficult to distinguish from brown or tan stains produced from liquids or suspensions. Similarly, it can be challenging to determine the type of fiber present in a sample turned to ash. This study offers insight to the use of ATR/FTIR to answer such questions.

A salient aspect of this study is the evaluation of spectral characteristics when multiple layers of different fabrics were burned together. This occurrence would be commonly expected in routine case work where layered fabrics are burnt together; deposits or transfer of aerosol products produced by burning cellulosic fabrics may also occur. This work describes the spectral characteristics of two fabrics in contact with each other subjected to various stages of heat damage and identifies numerous changes depending on fabric types.<sup>4</sup>

Ongoing studies are being performed to create a comprehensive understanding of spectral changes specific to various stages of heat damage and fabric type; the results will be presented in a tabular format. This work includes the creation of a spectral library of fabric that includes entries pre- and post-heat damage.

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**Forensic Fabric Evaluation, Heat Damage, ATR/FTIR**

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**E80 A Quantitative Analysis of Smokeless Powder Particles in Post-Blast Debris Via Gas Chromatography/Vacuum Ultraviolet Spectroscopy (GC/VUV)**

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**Learning Overview:** Attendees of this presentation will gain information about post-blast debris analysis via GC/VUV and the importance of the subsequent quantification of components present in post-blast smokeless powder particles.

**Impact Statement:** This presentation will impact the forensic science community by presenting information about the VUV spectrometer and the ability to utilize unique internal standards for the quantification of explosive components in post-blast debris.

The goal of this research is to quantify nitroglycerin, 2,4-dinitrotoluene, diphenylamine, ethyl centralite, and dibutyl phthalate in post-blast smokeless powder particles using heptadecane as an internal standard. An internal standard solution of 100ppm heptadecane in acetone was made and used as the solvent for all experiments.

Post-blast debris was obtained via controlled explosions with assistance from the Indiana State Police Bomb Squad. Two galvanized steel and two Polyvinyl Chloride (PVC) Improvised Explosive Devices (IED) were assembled. For both materials, one device contained IMR 4064 (single-base smokeless powder) and the other contained Alliant Red Dot (double-base smokeless powder). Each device was placed in a steel box to help retain the post-blast debris for collection.

Intact particles were extracted from debris using forceps and an Olympus® SZ61 stereomicroscope equipped with a Fiber-Lite MI-150 High Intensity Illuminator. Solutions containing intact powder were prepared at 1,000ppm in internal standard solution. These solutions were then filtered using a Polytetrafluoroethylene (PTFE) filter. Calibrants were prepared and analyzed on the same day as the post-blast samples to ensure reproducible and reliable quantification.

Each sample was injected into an Agilent® 7890B GC equipped with an Agilent® 7693 autosampler and a multimode inlet. The sample was introduced using hydrogen carrier gas at 2.4mL/min. The multimode inlet was in splitless mode with a temperature program beginning at 50°C and ramped to 200°C at 900°C/min. The analytes were then separated by a 15m x 320µm x 0.25µm Restek Rtx®-5MS column. The oven temperature began at 50°C, was held for 30 seconds, and was then ramped at 20°C/min to 250°C. The same oven temperature program was used for all samples. After separation, analytes were introduced into a VUV Analytics® VGA-101 VUV spectrometer at a transfer line and flow cell temperature of 300°C. Samples were analyzed using a scan rate of 4.5Hz, a deuterium lamp at 1.2V, a make-up gas pressure of 0.00psi, and a spectral range of 120–430nm. To improve the signal-to-noise ratio, the resulting chromatograms were analyzed using data from 125–240nm.

2,4-dinitrotoluene and diphenylamine were successfully quantified in the single-base smokeless powder post-blast debris while nitroglycerin, diphenylamine, and ethyl centralite were successfully quantified in the double-base smokeless powder post-blast debris. Compounds were detected as low as 7µg of 2,4-dinitrotoluene per mg of intact powder, <3µg of diphenylamine per mg of intact powder, 113µg of nitroglycerin per mg of intact powder, and <3µg of ethyl centralite per mg of intact powder in all devices. The concentration of 2,4-dinitrotoluene in IMR 4064 post-blast debris originating from the steel IED is lower than debris originating from the PVC IED. This could possibly be due to the steel IED reaching higher temperatures, leading to the increased consumption of 2,4-dinitrotoluene.

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**Vacuum Ultraviolet Spectroscopy, Forensic Explosive Analysis, Gas Chromatography**

## E81 Addressing the Quantitative Aspect of Single Grain Forensic Palynology

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**Learning Overview:** After attending this presentation, attendees will have gained an understanding of a non-destructive genomic analysis application of single pollen grains by combining the techniques of quantitative Polymerase Chain Reaction (qPCR), flow cytometry, and microscopy.

**Impact Statement:** This presentation will impact the forensic science community by providing a simplified and quantitative pollen grain analysis by combining two affordable techniques and their protocols for forensic labs at the state or local levels.

The ability to identify a plant species through pollen samples has played a role in various technological, industrial, and scientific fields throughout the years.<sup>1</sup> For example, airborne pollen monitoring to manage seasonal respiratory diseases, food quality control of honey, plant-pollinator interactions, and forensic botany.<sup>1-7</sup> However, pollen studies are limited by methods that are labor-intensive, time-consuming, and prone to misidentification.<sup>8</sup> As a result, there is a growing need for quick and effective pollen identification that allows for quantification.<sup>8</sup> The two most used methods for pollen identification are microscopy and DNA metabarcoding.

Microscopy is the gold standard for quantitative and qualitative pollen analysis.<sup>8</sup> Pollen assemblages, consisting of hundreds of grains, are typically stained, and morphology is determined under a microscope.<sup>7,9</sup> However, regardless of the field, pollen identification relies on expert knowledge examining the samples and high manual effort leading to a considerable cost, time-consuming, and low sample throughput.<sup>1,4,8,10</sup> Another disadvantage to this method is that identification can only occur at the family level or higher for many species and may fail to detect rarer species.<sup>1,11</sup> This identification level is because pollen grains from the same family cannot be differentiated due to their shared morphological features.<sup>2</sup> In recent studies, flow cytometry has been presented as an alternative optical method for counting and identifying pollen grains.<sup>8</sup> In addition, it allows for higher capture rates than traditional microscopy.<sup>8</sup> This study compares the conventional microscopy method with flow cytometry for pollen identification based on scattering and fluorescence characteristics on a single grain level. The combined data obtained from single grains opens the door for other downstream applications, including plant species identification at the individual grain level in soil, air collection, and clothing swabs samples.

DNA metabarcoding identifies large quantities of pollen samples at the species level using standardized barcode marker gene regions.<sup>8</sup> To date, five genetic markers have been used for DNA barcoding and metabarcoding: *matK*, *rbcL*, ITS2, *trnH-psbA*, and *trnL*.<sup>4</sup> One standard method to determine one species from another is through sequence variation using Polymerase Chain Reaction (PCR).<sup>12</sup> However, PCR alone cannot quantitatively assess the amount of DNA present at the beginning of the process before amplification, and qPCR is used in this respect.<sup>12</sup> Quantitative methods are commonly based on calibrations curves, either using threshold cycles as a proxy or initial seed values from amplification models of the experimental curves, as a function of the logarithm of DNA concentration.<sup>13</sup> This study compares these approaches. QPCR analysis of the chloroplast genetic markers *rbcL* and *matK*, the second nuclear ribosomal ITS2 region, was used for each plant species. The species evaluated were *Ambrosia artemisiifolia* (common ragweed), *Paspalum notatum* (bahiagrass), *Artemisia vulgaris* (common mugwort), *Quercus virginiana* (southern live oak), *Pinus echinata* (shortleaf pine), *Taxodium distichum* (bald cypress), *Phalaris arundinacea* (reed canary grass), and *Plantago lanceolata* (ribwort plantain).

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### DNA, Pollen, Palynology

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## E82 Chemometrics in Forensic Examination

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**Learning Overview:** Attendees will learn about the type of chemometric methods that can be utilized in the analysis of trace evidence. This type of data analysis from different types of samples will be shown.

**Impact Statement:** Chemometrics is known to have a lot of potential in becoming an effective tool for analyzing the data and reaching objective conclusions with significant results. The past decade has seen the application of the chemometric methods combined with analytical techniques for characterization, and discrimination of samples, which leads to the informative and representative examinations of the samples. This presentation has been divided into various sections that include chemometrics, its history, supervised/unsupervised methods, and the application of chemometrics in various disciplines of forensic science. It is suggested that these new techniques and mathematical/statistical methods are having statistical significance and hence are highly suitable for forensic casework.

Forensic science can broadly be defined as the science successfully applied in legal matters. It involves the careful examining of complex evidence that would be helpful in solving various crimes. The correct identification of the samples along with their classification, per the class characteristics, are a crucial step in forensic science as it will be helpful in forming an unbiased verdict in legal cases. The focus of every forensic investigation is the establishment of linkage between places, objects, or people for the reconstruction of events that occurred in any crime. This is typically done through recovery, analysis, and interpretation of physical evidence. However, the recovery and analysis of physical evidence is not the conclusive stage, as many problems occur at the level of interpretation of results. Numerous disciplines in forensics are dependent upon the visual comparison of multivariate data available in the form of spectra, chromatograms, or other analytical output. The comparison and interpretation require an expert to handle the complex data in order to provide a valuable conclusion or information. In addition, it is always mandatory that the evaluative information must be presented in a way that it is understood by the courts. The subjective nature of these comparisons has led to concerns regarding potential human error and cognitive bias. Although this issue has been of relevance to pattern analysis, it also relates to the “eye-balling” of chemical data across a wide range of forensic analyses. This was highlighted in a broadly publicized Report by the United States National Academy of Sciences (NAS) in 2009, which determined that more rigorous protocols were required to guide the subjective interpretations relied upon within many forensic disciplines.<sup>1</sup> Following the publication of the NAS Report, as well as a similar inquiry conducted by the British House of Commons Science and Technology Committee, there have been strengthened efforts to establish statistically validated bases for assessing evidential value.

One such approach is the use of chemometrics, a discipline that employs well-established statistical protocols to model chemical data. The employment of statistical methods eventually strengthens the validity of the evidence as it is described in probabilistic terms. The major goal of forensic science lies in providing appropriate methods of analysis in crime investigation for obtaining suitable evidence, successful investigation, and fair court decisions. In recent years, various problems have been worked on by this study pertaining to the analysis of trace evidence in forensic science using chemometrics. The types of evidence that were analyzed by this study included kohl, lipstick stains, soil, kohl stains, paper types, cling films, cosmetics used by women, and wood samples. The results demonstrated that the use of chemometrics provide more reliable and validated results with 99.9% discrimination among the samples. Also, category classification is possible utilizing supervised chemometric methods. Therefore, chemometrics is known to have vast potentiality in becoming an effective tool, but there are still some gaps between it and forensic analysis. Owing to the high variety of situations and sample variation found in forensic casework, expertise with the chemometric methods applied is essential to ensure the generation of meaningful results and avoid erroneous conclusions.

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- <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>.

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### Chemometrics, Trace Evidence, Multivariate Analysis

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## E83 Spectrally Based Color Calibration of Microscopes for the Analysis of Birefringence

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**Learning Overview:** After attending this presentation, attendees will understand the importance of color calibration and the advantage of using appropriate color spaces to quantify fiber birefringence determination.

**Impact Statement:** This presentation will impact the forensic science community by providing a quantitative methodology for calibrating the color spaces in microscopy and using it for a more accurate birefringence determination of fibers.

In forensics, textile fibers are one of the most common pieces of trace evidence found at a crime scene. This provides a way to connect an assailant to a crime scene. The quantitative analysis of fibers is important to the growth of improving trace analysis techniques. Fibers are anisotropic, allowing for the determination of birefringence using interference colors. Each fiber has a range of possible birefringence values dependent on thickness that cause true identification to be difficult. Color analysis is crucial in being able to discriminate between fibers found at a crime scene. These colors can be used to estimate the birefringence range of a fiber. Typically, color is analyzed using a color space. The most common color spaces include RGB, XYZ,  $L^*a^*b^*$ , and  $L^*u^*v^*$ . Unfortunately, color is very subjective and often difficult to quantify accurately, even using digital detectors. Finding true matches of colors has been limited by factors including illumination, optics transmission function, and, in many cases, the human eye. The colors produced by the birefringence of fibers under analysis using Polarized Light Microscopy (PLM) are known to provide a quick information on the fiber identification. Nonetheless, the exact determination of the accurate colors is still either subjective to the operator or the correct calibration of the software.

A variety of textile fibers (nylon, rayon, acetate) were analyzed for birefringence using a PLM. Calibration of the color response of the microscope camera was performed using a Ultraviolet/Visible (UV-Vis) broadband spectrometer used to determine the CIE XYZ 1931 reference values for the illuminant. All the color images obtained with the camera were then transformed from the RGB color space using MATLAB® into  $L^*a^*b^*$  color space using the references of the illuminant of the microscope just measured above. An empirical Michel-Levy chart was also designed using a quartz wedge in order to calibrate retardation with empirical  $L^*a^*b^*$  color values.

The discriminatory power and uncertainty of color perception of the microscope camera and of human vision were measured and will be discussed in this presentation.

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### Microscopy, Color, Calibration

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**E84 The Persistence of Diatoms as Trace Evidence in Clothing Fabrics: The Effect of Active Removal (Machine Washing) and Passive Removal (Time and Environment)**

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**Learning Overview:** After attending this presentation, attendees will understand newly explored factors that influence the persistence of diatoms as a form of physical trace evidence within different variations of clothing fabrics when subjected to environmental removal pressures and settings. This presentation will increase attendees' awareness of diatoms, their potential applications, and their limit as trace evidence and factors affecting its role as such.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of the use of the diatoms in forensics, specifically as trace evidence, as well as exploring aspects of their persistence in common clothing fabrics in practical contexts.<sup>1,2</sup>

Aquatic crime scenes present a challenging environment for crime scene investigators to operate in. Generally, many conventional forms of evidence that are relied upon in terrestrial environments, such as trace evidence like fibers or hairs, are unavailable in aquatic environments due to their ephemeral nature and aquatic life therein. However, aquatic environments are host to organisms that can have evidential use in the absence of more traditional evidence. Diatoms are microscopic algae with cell walls composed of silica, initially largely used to aid in drowning diagnoses. More recent literature has shifted the attention of diatoms in forensics from the area of forensic pathology to that of trace evidence.<sup>3,4</sup> So far, the research mostly centers on diatom transfer from environments to clothing and their subsequent extraction from clothing fabrics for analysis.<sup>2,5</sup> Little research has yet been published concerning the persistence of diatoms in clothing fabrics.

The aim of this study is to analyze the effects that various active (manual) and passive (natural) removal pressures have on the persistence of diatoms within various common clothing fabric of different constructions and compositions (pure cotton knit, denim weave, pure polyester knit). Fabrics were submerged in Diatom-Enriched Waters (DEW) prepared using 50:50 mixed cultures of native and common Australian diatom species (*Chaetoceros muelleri* and *Navicula* sp.).

In the active experiment, the fabrics were washed in washing machines (top loader and front loader) at varying temperatures (cold and warm >40°C) for one, two, and three consecutive washes, to assess the persistence of diatoms in the fabric samples when subject to an attempt to remove them via machine washing actively. Additionally, some unsubmerged fabric samples were introduced to the washes to assess whether cross-contamination could occur.

In the passive experiments, the fabrics were left in various environments (indoors and outdoors) and sampled at increasingly longer time intervals for three months to assess any passive reduction due to natural passive pressures without any active influence.

This presentation will discuss the outcome of this study and provide interpretations and implications of the results in the light of future case work.

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**Diatoms, Persistence, Forensic Trace Evidence**

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## E85 An Assessment of Swab Efficiency for Body Fluid Identification and DNA Isolation and Analysis in Different Types of Fabric

*Gabriela Roca, PhD\**, SERATEC, Goettingen, Lower Saxony, Germany; *Sara C. Zapico, PhD*, New Jersey Institute of Technology, Newark, NJ and Smithsonian Institution, Washington, DC

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**Learning Overview:** After attending this presentation, attendees will understand the importance of swab types not only for efficient DNA isolation and analysis, but also for body fluid identification.

**Impact Statement:** This presentation will impact the forensic science community by introducing, for the first time, the simultaneous assessment of body fluid identification, DNA isolation, and characterization, taking into consideration the type of swab and fabrics, which will lead to applying these techniques in crime scene investigations.

Recovering appropriate evidence from crime scenes to identify perpetrators or in certain cases, the victim, is one of the main goals of forensic investigation. As a result, identification of body fluids could be crucial toward this purpose. Current techniques of body fluid identification include, among others, the use of Alternative Light Sources (ALS), chemical assays, and/or immunochromatographic tests. The latter are based on the specific antigen-antibody reaction against a human protein, with higher expression in the body fluid in question. These tests are simple, quick, and suitable to use at crime scenes.

As with any other tests, the first step to apply these immunochromatographic tests is to retrieve the potential sample with a swab moisturized in a specific buffer. The swabs normally used at crime scenes are cotton swabs. However, there are other types of swabs available in the market that have been proven to be efficient for subsequent DNA isolation and analysis.

The aim of this study is to assess the efficiency of different types of swabs for body fluid identification applying immunochromatographic tests, as well as DNA isolation and characterization.

Fifty microliters of human saliva were deposited in three different types of textiles (denim, cotton, and polyester). Three replicates per each swab type and fabrics were evaluated. After 24 hours at room temperature, saliva samples were recovered applying three different types of swabs: cotton swabs, COPAN® 4N6FLOQSwabs™ Regular size tip, and COPAN® 4N6FLOQSwabs™ Subungual Shape. Swabs were incubated in agitation in 300ul of extraction buffer from Seratec® SALIVA CS test, during 10 minutes. After that, three drops of buffer were added to the Seratec® SALIVA CS immunochromatographic test, and the results were recorded.

Total DNA was isolated from the extraction buffer using silica-based columns, and later quantified applying fluorescent methodology. Promega® PowerPlex® Fusion 6C System was used to amplify and characterize 23 autosomal Short Tandem Repeats (STRs) and Amelogenin gene in 1ng of DNA. Mitochondrial DNA (mtDNA) characterization was carried out using BigDye™ Direct Cycle Sequencing kit for Hypervariable Regions I and II. Statistical analyses were carried out in SPSS® Statistics version 26.

Immunochromatographic test results showed differences among swab types. In denim and cotton, band intensities are higher with 4N6FLOQSwabs™ Regular size tip and 4N6FLOQSwabs™ Subungual Shape than with cotton swabs. In contrast, polyester showed the same intensities in the three types of swabs.

DNA concentration showed the same pattern, obtaining higher yields of DNA with 4N6FLOQSwabs™ Regular size tip and 4N6FLOQSwabs™ Subungual Shape in denim and cotton, and similar concentrations with the three types of swab in polyester. A two-way Analysis of Variance (ANOVA) demonstrated that both type of swabs and fabrics, individually and together, influenced DNA yield.

After DNA profiling, three parameters were assessed toward determining DNA quality: summary of peak height, Mean Local Balance, and Profile Index, according to a previous publication. After calculation of these parameters and application of two-way ANOVA, in this case, only the swab type had an impact on DNA quality.

Findings from this research provide evidence that the swab type could be crucial to efficiently identify body fluids as well as increasing the DNA yield and quality in forensic samples.

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### Swab, Body Fluid, Saliva



## E86 An Elemental Analysis of Bullets Via Atomic Absorption Spectroscopy

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**Learning Overview:** This presentation will address current issues with firearm and tool mark analysis while providing a scientific solution that is understandable to those in various fields.

**Impact Statement:** This research is beneficial to the forensic science community due to the necessity of scientific-based evidence implemented in court along with the opportunity for increased efficiency in criminal investigations.

Firearm identification is a vital element in thousands of crime scene investigations per year. As violent crimes involving firearm use increase, the need for accurate and scientific conclusions with quickly obtainable results increases as well.

Current modes of analysis have caused controversy in the legal system due to the subjectivity and non-scientific basis of the firearm and tool marks examiner's conclusions on whether projectiles found are a match to the suspected weapon used. Examiners base their findings on microscopic evaluations of markings found on the projectile. These observations are then compared to test-fired rounds and leads the examiner to four possible conclusions, which are identification, inconclusive, elimination, or not suitable for comparison. It is argued that evidence based on observation only is not sufficient as many individuals view objects differently.

A method in which a bullet can be identified without the recovery of the projectile or firearm at a crime scene has not been developed nor has scientific-based analysis of ballistic evidence been implemented in criminal investigations prior.

The aim of this project is to solve these current issues by introducing a new method of ballistic analysis via Atomic Absorption Spectroscopy (AAS). This instrumentation allows for the detection of various elements present in bullets. More specifically, the ratios of metal concentrations, which has shown to be unique to manufactures and types of bullets from the results of this research so far. Forensic scientists can accomplish this method with or without discovery of casings or bullets on the scene. The metal deposits created by contact between a surface and the bullet can be extracted, so there is no need for the physical projectile to identify the firearm in cases where the weapon is missing. Another goal of this research is to develop a database of these ratios specific to each manufacturer to determine the bullet type and brand for reference when comparing AAS results.

Implementation of this technology can be beneficial to these cases involving firearm use in many aspects. This scientific approach adds probative value to the expert witness' testimony in court to corroborate their statements along with decreased inconclusive or unsuitable for comparison conclusions. AAS instrumentation only requires a very small sample volume, which provides a solution for fragmented samples that fall under the category of the unsuitable for comparison conclusion. Additionally, damaged surfaces can serve as a replacement for the absence of ballistic evidence rather than simply having a missing piece of evidence that is important to solve the investigation.

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### Crime Scene Investigation, Forensic Ballistics, Atomic Absorption Spectroscopy

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**E87 A Pre-Post Experimental Study on the Life of Blood Stains on Different Soil Samples**

*Eleena Philip, MS\*, University of Mysore, Abu Dhabi, UAE*

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**Learning Overview:** After attending this presentation, attendees will be familiar with an experimental study to qualify and quantify the degree of degradation of various bloodstains at various temperatures on commonly found environmental soils.

**Impact Statement:** Most of the time, blood found at a crime scene is in a very small quantity though its potency is very high. If blood is affected by any circumstances, then it is difficult to identify the individual or a person involved in a crime. Blood may be found on any type of soil either indoor or outdoor. So, the study signifies the time period up to which blood grouping can be done in such cases and also the time period up to which blood can be retained as such before the process of degradation. If the results obtained from the present study are in line with expected results, then the same can be of great significance for the purpose of forensic investigations.

Biological materials, especially blood stains, that may be found at crime scenes are subject to degradation because of external environmental factors, including contaminants, heat, and humidity. Hence, the integrity of such samples may result in partial or even no significant values, which could challenge forensic investigations. Most research has focused on the DNA quantification on various surfaces, but only a few researches focused on the relationship between blood and soil.<sup>1,2</sup> In this study, an attempt was made to qualify and quantify the degree of degradation of various bloodstains at various temperatures on commonly found environmental soils. For this study, human blood from each blood group was collected and deposited on various predetermined soil types and stored in a controlled environment at different temperatures over a period of 20 days. Each soil type was analyzed and recorded for their Ph and micronutrient content prior to the experiment. The samples were analyzed using absorption elution and the technique was combined with Ultraviolet (UV) spectroscopic analysis. As blood undergoes physical and biochemical changes over time, the changes are analyzed by the characteristic absorptions in the UV-visible spectral region. The overlay UV-visible spectrum of the blood over time in various soil types was recorded at six peaks in correspondence with the constituents of blood. The results demonstrated that although the type of blood group had no significant value in the process of degradation, the temperature and soil type revealed a drastic change in the quality of blood stains. Moreover, the micronutrients present in each soil type had a direct relationship with blood degradation over time. Furthermore, each amino acid in blood was analyzed separately. Initial examination after day 5 showed a significant decrease in certain compounds, while others remained stable. But in due time, each compound showed a different value in relation with quantity with regard to the various soil type.

**Reference(s):**

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**Blood, Soil, Experiment**

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**E88 Investigation Into the Aging Mechanism of Bloodstains Post-Deposition Using Steady-State Fluorescence Spectroscopy for Forensic Purposes**

*Alexis Weber, MSFS\*, SupreMEtric, Albany, NY; Anna Wójtowicz, BS, Jagiellonian University, Kraków, Poland; Igor Lednev, PhD, University at Albany, SUNY, Albany, NY*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the biological aging mechanism of peripheral and menstrual bloodstains.

**Impact Statement:** This presentation will impact the forensic science community by presenting information on the fundamental aspects of blood aging.

Blood is one of the most common body fluids discovered at crime scenes involving violent actions.<sup>1</sup> It is considered one of the most important types of forensic evidence since it allows for the identification of the individual, providing that there is a match with a known DNA profile.<sup>2</sup> However, there is additional information that could be learned from bloodstains. Determining the Time Since Deposition (TSD) could further assist investigators by establishing when the crime occurred or if a bloodstain present is related to the investigated event. Additionally, if crime scenes contain multiple sets of bloodstains, the TSD determined for individual bloodstains should allow for the selection of bloodstains relevant to the crime and, therefore, reduce the number of samples that should be collected, documented, and processed, thus creating a more efficient workflow within the forensics community.

To develop a forensically sound method for determining the TSD of a bloodstain, it is necessary to understand the underlying biochemical mechanisms occurring during aging. As biochemical processes occurring in blood are necessary for the continued survival of living organisms, they are important subjects of human biology and biomedicine and are well understood. However, during a violent criminal event where bleeding occurs, the blood will be deposited onto a surface. The biochemistry of bloodstain aging *ex vivo* is primarily of interest to forensic scientists as it has not yet been thoroughly researched specifically.

This preliminary study utilizes steady-state fluorescence spectroscopy to probe and compare the changes in fluorescence properties of peripheral and menstrual blood up to 24 hours post-deposition.<sup>3</sup> It was shown that peripheral and menstrual blood exhibited similar kinetic changes over time, assigned to the presence of the fluorophores: tryptophan, Nicotinamide Adenine Dinucleotide (NADH), and flavins in both biological fluids. The unique biochemical mechanisms of blood aging *ex vivo* will be discussed.

**Reference(s):**

1. Li, R. *Forensic Biology*. Second Edition ed.; CRC Press Taylor & Francis Group: Boca Raton, FL, 2015.
2. Virginia Department of Forensic Science, *Forensic Biology Section Procedures Manual*, Section II, Presumptive and Confirmatory Tests for Biological Substances. 2014; Vol. 4.
3. Weber, A.; Wójtowicz, A.; Lednev, I.K. Post deposition aging of bloodstains probed by steady-state fluorescence spectroscopy. *Journal of Photochemistry and Photobiology B: Biology* 2021, 221, 112251.

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**Fluorescence Spectroscopy, Aging Mechanism, Blood Analysis**

## E89 A Membrane-Modulated Centrifugal Microfluidic Platform for Field-Forward Immunodetection of Opioids

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**Learning Overview:** After attending this presentation, attendees will have been introduced to a novel microfluidic method for antibody-based detection of opioids that leverages an Enzyme-Linked Immunosorbent Assay (ELISA) with colorimetric readout.

**Impact Statement:** This presentation will impact the forensic science community by presenting a cost-effective, portable method for immunodetection of forensically relevant illicit and misused substances.

Opioids are a common class of illicit or misused substances and currently cause the majority of overdose-related fatal intoxications.<sup>1,2</sup> Such widespread use has exposed the need for accurate, reliable Point-of-Need (PoN) presumptive drug tests to produce actionable information on-site to inform an appropriate, timely response. However, many colorimetric forensic field tests are subjective and non-specific; spectroscopic techniques are often prohibitively expensive.<sup>3</sup> As a result, many jurisdictions forgo field testing altogether, instead shipping seized samples directly to centralized laboratories.<sup>4</sup> Immunoassays, including ELISAs, serve to presumptively identify substances prior to confirmatory spectroscopic analysis, but these methods are traditionally time-consuming and labor-intensive, requiring substantial manual sample handling by trained analysts. Such delays in obtaining accurate results can have markedly negative impacts on public prevention efforts attempting to avoid adverse public outcomes; there is a need for fast, reliable detection methods.

The goal of this work is to adapt ELISA workflow to a portable, centrifugal microfluidic platform that automates and expedites the assay for reliable, accurate opioid detection by non-technical personnel. Broadly, microfluidic strategies provide several advantages over traditional laboratory methods, including minimized sample and reagent consumption, decreased cost-per-test, ease of automation, enclosed formats, and shortened analytical intervals. Among these, centrifugal microfluidics is especially appealing given that simple, rotationally controlled fluid flow eliminates the need for bulky external equipment (e.g., pumps, to ensure portability).<sup>5</sup> This study leverages this method to streamline ELISA workflow and permit strict adherence to the stepwise protocol needed for successful immunodetection. Incorporation of colorimetric output, and an associated objective interpretation method, ultimately permitted simultaneous, parallel detection of opioids from eight samples in as little as one hour—a marked decrease in required analytical time relative to the traditional multi-day timeline.

Optimized microarchitecture included embedded membrane microvalves to enhance on-disc fluidic control; at low rotational frequencies, fluid was temporally retained in the immunodetection chamber for incubation and/or mixing, whereas high-speed disc rotation drove flow through the microvalve and out of the chamber. On-disc chromogenesis was comparable with parallel traditional, plate-based ELISA results. Morphine and a key metabolite were detected in the ng/ $\mu$ L range, in buffer and artificial urine, respectively. Assay specificity was demonstrated through differentiation of opioids from several other drug classes with no observed cross-reactivity with common adulterants found in seized street-level substances. Together, these results highlight the broad potential application of this technology, spanning from use by law enforcement officers for roadside drug testing to toxicological urinalysis.

Future work involves integration of on-board reagent storage and parallel, simultaneous screening for multiple substances. The work described represents significant progress toward a true “lab-on-a-chip” device for affordable, simple, *in situ* drug testing.

### Reference(s):

1. Scholl, L.; Seth, P.; Kariisa, M.; Wilson, N.; Baldwin, G. Drug and opioid-involved overdose deaths—United States, 2013–2017. *Morbidity and Mortality Weekly Report* 2019, 67 (51-52), 1419.
2. UNODC, World Drug Report 2020. *Global overview of drug demand and supply*. 2020, 2.
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5. Gorkin, R.; Park, J.; Siegrist, J.; Amasia, M.; Lee, B.S.; Park, J.M.; Kim, J.; Kim, H.; Madou, M.; Cho, Y. K. Centrifugal microfluidics for biomedical applications. *Lab on a Chip* 2010, 10 (14), 1758-1773.

### Opioids, Enzyme-Linked Immunosorbent Assay (ELISA), Microfluidics

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## E90 Non-Contact Detection of Fentanyl by a Field-Portable Ion Mobility Spectrometer

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**Learning Overview:** After attending this presentation, attendees will understand how a non-contact detection method for fentanyl was developed.

**Impact Statement:** This presentation will impact the forensic community by providing a non-intrusive and safer way for the detection of fentanyl and fentanyl-related substances.

Fentanyl is a potent synthetic opioid that has vast medical benefits. This Schedule II drug is approved as an analgesic for chronic pain, used as a general anesthetic, and is often given to women during childbirth. These medical benefits have been outweighed by the increased illicit use of fentanyl. At the height of the COVID-19 pandemic, there was an acceleration in deaths caused by opioids, with over 81,000 documented overdoses with illicitly manufactured fentanyl as one of its leading forces.<sup>1</sup> Inhaling mere milligrams of fentanyl can result in an overdose, which is not only dangerous to the user, but also law enforcement. To prevent exposure, the National Institute for Occupational Safety and Health (NIOSH) recommends that the scene be handled by specifically trained personnel wearing proper protective equipment.<sup>2</sup> The ability to safely detect fentanyl is imperative for first responders and law enforcement.

Currently, there are several ways to detect fentanyl in the field. However, all require direct sampling of bulk material, which puts the technician at risk. To date, there have been very few non-contact methods developed for the detection of fentanyl. A method using fentanyl vapor would provide a non-invasive and non-destructive manner of detecting fentanyl. Unfortunately, fentanyl has a low vapor pressure, making it virtually undetectable directly in the vapor phase. Conversely, by determining the vapor signature of fentanyl, the target analytes can be identified and used for detection in the vapor phase. Solid Phase Microextraction with Gas Chromatography/Mass Spectrometry (SPME-GC/MS) was used to identify the target analytes from the vapor signature of pharmaceutical-grade fentanyl, fentalogs, and street-grade fentanyl. Comparative analysis of the vapor profiles indicated two target analytes: N-Phenylpropanamide (NPPA) and N-Phenethyl-4-Piperidone (NPP). These target analytes can be used to detect fentanyl in the vapor phase using a portable vapor detector.

Hand-held Ion Mobility Spectrometers (IMS) offer a sensitive, user-friendly, and quick method for narcotics and explosives detection. This presentation describes the method developed for detection of target analytes for fentanyl using a handheld IMS. First, the drift times were determined to be 5.896ms for NPPA and 6.484ms for NPP. The drift times were then used to program alarms in the handheld IMS system. Finally, the two alarms were tested by sampling the vapors of NPP, NPPA, pharm-grade fentanyl, and adulterated pharm-grade fentanyl samples. The system was able to accurately identify the target analytes when sampling NPP and NPPA. Both analytes were found in pharm-grade fentanyl, while only NPPA was found in the adulterated pharm-grade samples. Common adulterants and over-the-counter drugs were tested and none alarmed for NPP or NPPA. Limit of detection was tested using two methods and determined to be as low as 5ng. Further development of the system is being conducted to accurately identify street-grade samples.

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### Vapor Detection, Fentanyl, Ion Mobility Spectrometry

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## E91 Sweat Analysis Leading to Possible On-Site Detection for Drugs and Alcohol in the Body

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**Learning Overview:** After attending this presentation, attendees will have a deeper understanding of the practicality and applications of colorimetric, non-invasive, on-site drug detection systems.

**Impact Statement:** This presentation will impact the forensic science community by introducing a novel, non-invasive sensing system that has the ability to transform the model of drug and alcohol testing in the field for law enforcement personnel.

As society advances, the advancement of analytical detection methods for illegal substances and their metabolites must advance in a parallel fashion. As such, there is a need for accurate, rapid testing methods for drugs and alcohol for medical purposes as well as for law enforcement personnel in the field.

The current technology used for testing bodily fluids for drugs and alcohol requires invasive sampling of blood or urine, as well as a laboratory with trained staff for analysis of the samples. The need for laboratory testing creates a time-related bottleneck for the return of results to the requesting parties, as well as introducing more room for error as the submitted sample ages between collection and analysis. This process can potentially lead to delaying any legal proceedings that depend on the accurate analysis of the sample.

Particularly, the detection methods for Blood Alcohol Content (BAC) are outdated, as alcohol has been legal to consume for decades across the country and current on-site BAC testing methods can still only be used as preliminary evidence for suspected operation of machinery or Driving While Intoxicated (DWI) charges.<sup>1</sup> Additionally, the recent legalization of marijuana for medical and recreational use in many states has led to a push for on-site testing capabilities to determine if a person is under the influence of the psychoactive component of marijuana, Tetrahydrocannabinol (THC), at the time of questioning under what is likely a zero-tolerance use policy.<sup>2</sup>

The aim of this research is to develop a non-invasive sensing concept for targeting and quantifying certain substances of interest in sweat, as it is easily collected from most individuals in a non-invasive manner. Two concepts for non-invasive sensing in sweat have been developed: one for ethanol quantification relating to BAC, and one for THC metabolite detection.<sup>3,4</sup> These methods utilize enzymatic and immunoassay components, are non-invasive in nature, and provide colorimetric feedback. This method of result reporting provides an opportunity for advancing these systems to on-site detection via handheld Ultraviolet (UV) -visible spectrophotometry devices and even smart phones with specialized software for colorimetric analysis via the built-in camera.

This technology could pave the way for a simple, on-site visual test for multiple kinds of drugs and alcohol for law enforcement and medical staff to operate in that moment of need, instead of submitting the individual involved to traveling to a facility, having blood samples drawn for laboratory testing, and losing valuable time as the body continues to metabolize these compounds.

### Reference(s):

- <sup>1</sup> *What is Blood Alcohol Level Evidence?* (n.d.). Retrieved from <https://www.acecriminalattorneys.com/dui-defense/what-is-blood-alcohol-level-evidence>.
- <sup>2</sup> Dills, A.K.; Goffard, S.; Miron, J. (2017). *The effects of marijuana liberalizations: Evidence from monitoring the future* (No. w23779). National Bureau of Economic Research.
- <sup>3</sup> Brunelle, E.; Thibodeau, B.; Shoemaker, A.; Halámek, J. (2019). Step toward roadside sensing: Noninvasive detection of a THC metabolite from the sweat content of fingerprints. *ACS sensors*, 4(12), 3318-3324.
- <sup>4</sup> Hair, M.E.; Gerkman, R.; Mathis, A.I.; Halámková, L.; Halámek, J. (2019). Noninvasive concept for optical ethanol sensing on the skin surface with camera-based quantification. *Analytical chemistry*, 91(24), 15860-15865.

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### Non-Invasive Sensing, Sweat Analysis, Drug Detection

## E92 A Multidisciplinary Forensic Approach: The Investigation of a “Kuman Thong”

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**Learning Overview:** The goal of this presentation is to underline how, in unusual and complicated cases of forensic interest, a multidisciplinary approach is pivotal in order to reach a solution.

**Impact Statement:** This presentation will impact the forensic science community by reporting an unusual case concerning the identification of a human-like amulet and describing the multidisciplinary approach adopted to define its nature and the species of belonging.

In the world of esotericism, some artifacts may reproduce human parts or features. Most difficulties concern the determination of their nature and, if real, whether they have an animal or human origin. In the present report, investigations carried out to determine the nature of a human fetus-like “Kuman Thong” are described.

In Thai culture, a “Kuman Thong” is a traditional amulet believed to bound the spirit of an unborn child, which, if properly revered, can bring prosperity and luck. According to tradition, Kuman Thongs were created from a human fetus.<sup>1</sup> The ritual included roasting the fetus at a cemetery and sometimes even soaking it in oil extracted from the skin of a person who died under violent circumstances. Once dried, the corpse was covered in lacquer and gold foil, from which derives the name Kuman Thong, which means literally “golden child.” Nowadays, Kuman Thongs are plastic or wooden statues shaped like a little boy or fetus.

In 2018, the owner of an unauthorized occult store was charged with holding illicit material. The police found a human fetus-like object contained in a jar filled with transparent liquid, decorated with jewels. The Kuman Thong was entrusted to specialists to define its origin. A multidisciplinary approach was applied, including forensic pathology, radiology, anthropology, and molecular biology.

Medical-legal macroscopic examination showed the body was tightly fixed in a fetal position, dressed in a cloth diaper. The body surface, with a stone-like texture, was almost entirely covered with lacquer and gold leaf. The corpse was also adorned with yantras (small tattoos) and little talismans. Numerous details on the body suggested it was not a sculpture; skull shape and body conformation were like those of a human being. Some uncovered portions of the skin were covered by soft hair and different human-like anatomical structures were recognized. A possible cause of death of the skeletonized fetus could not be identified.

Radiological analysis was performed by a Computed Tomography (CT) scans, using an MD Sensation 64 scanner, then a 3D reconstruction of images were obtained using Multiplanar (MPR) and Visual Tracking (VTR) method.

Internal organs were collected using a drill and the resulting powder was extracted using a silica-based automated method. The results of the following amplification of multiple Short Tandem Repeat (STR) markers contained in a commercial kit validated for human identification showed that no genetic profiles could be achieved from the samples; this outcome was probably due to a massive degradation of the genetic substrate subjected to physical or chemical modifications.<sup>2,3</sup> Further studies on the mitochondrial DNA extracted from tissue samples are currently ongoing using conventional Sanger and Next Generation Sequencing (NGS) sequencing techniques.

Radiological and anthropological examination were unable to identify the nature of the Kuman Thong. CT detected and recognized both a complete and articulated fetal skeleton and images compatible in structure and density with human organs; anthropological measurements also gave an evaluation of fetus biological age and gender: based on development of the dental buds and diaphyseal lengths, the age was estimated to be 26-27 gestational weeks; and the shape of the jaw was compatible with a female subject.<sup>4-7</sup>

### Reference(s):

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3. Cattaneo, C.; Porta, D.; Gibelli, D.; Gamba, C. Histological determination of the human origin of bone fragments. *J Forensic Sci.* 2009 May;54(3):531-3.
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### Kuman Thong, Multidisciplinary Approach, Postmortem Computed Tomography

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## E93 Automatic Class Characteristic Recognition in Shoe Tread Images

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**Learning Overview:** After attending this presentation, attendees will have a basic understanding of the usefulness of machine learning for image identification and how this can help bridge the gap between unfriendly numerical features and descriptors used by examiners in practice.

**Impact Statement:** This presentation will impact the forensic science community by introducing a method of automatic pattern recognition in shoe soles for forensic evidence purposes.

One of the fundamental problems in footwear forensics is that the distribution of class characteristics in the local population is not currently knowable. Surveillance devices for gathering this data are just half of the battle; it is also necessary to process the data gathered using these devices and identify relevant features.

Machine learning has pioneered many developments in artificial intelligence for forensic science over the past ten years, however, efficient and effective object detection methods are being implemented using region proposal networks to pinpoint an object's location in an image.<sup>1</sup> This has resulted in the formation of the Faster Region-based Convolutional Neural Network (R-CNN). This network is especially helpful in detecting features in images and, in the case of this research, images of the bottoms of shoes.

The goal behind this research is to be able to feed an image of a shoe sole to a fine-tuned network that will output a set of object proposals. For example, after inputting an image of the bottom of a shoe, the network will output the same image but with bounding boxes around pre-determined objects. The objects in practice are shapes (circles, rectangles, quadrilaterals, etc.) and logos (Nike®, Adidas®, Polo®, etc.). A completed basic R-CNN model will be able to extract and identify the presence of any logo or shape in a shoe sole and predict with an "X" percent score that the object in question is that object.

This presentation will describe progress made in automatic identification of relevant footwear features—brand, shoe size, and tread pattern elements, as well as complications which arise when combining machine learning algorithms with human-friendly features. Using transfer learning to connect pre-trained neural networks to newly gathered and labeled training data, this method bridges the gap between unfriendly numerical features and descriptors used by examiners in practice. The Faster R-CNN has the capabilities to extract and identify features of an image the same way a human brain can. It can separate the areas of an image into background and regions of interest and then, subsequently, label the content of that region of interest to confirm or challenge a similar labeling task of an examiner.

Leveraging both clean training data and "messy" data gathered from the local community using newly developed footwear surveillance devices, this study will present developments in footwear forensics that will enable examiners to testify as to the frequency of class characteristics in the local population in the very near future.

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### Pattern Recognition, Footwear, Machine Learning



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**E94 A Comparison of Dust Cover Impact Marks on 200 Consecutively Fired Cartridge Cases From a Saiga 7.62x39mm Rifle**

*Malory Molt, BS\*, Washington State Patrol Crime Lab, Seattle, WA; Rick Wyant, MS, Washington State Patrol Crim Lab, Seattle, WA; Brian Smelser, BS, Washington State Patrol Crime Lab, Seattle, WA; Sarah Fox, Washington State Patrol Crime Lab, Seattle, WA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how impact marks from the dust cover of a firearm imparted on cartridge cases have the ability to change over repeated firings, which may directly impact microscopic examination and a firearm examiner's ability to make an identification.

**Impact Statement:** This presentation will impact the forensic science community by presenting how over the life of a firearm, tool marks have the potential to change, directly affecting microscopic examination and comparison.

During the first half of 2017, a series of shootings occurred in the Seattle, WA, area that yielded over 200 cartridge cases during five months. Many of the cartridge cases found at the various crime scenes were identified to a recovered Saiga 7.62x39mm caliber, AK-47 type rifle. Comparisons of the breech face area markings of this rifle type have been historically challenging for examiners due to manufacturing marks present on ammunition, ammunition primer hardness, and lack of markings on the firearm. Cycling marks related to the ejector and dust cover impact mark areas are routinely utilized to supplement identification.

Several studies have been reported on the marking longevity of many types of firearms and surfaces, including chamber marks and breech face marks on pistols and revolvers. These studies consistently demonstrate that while some slight changes may occur over many firings, marks on cartridge cases are not altered beyond identification. However, none of these reported studies considered dust cover impact marks. During this investigation, questions arose related to the persistence and reproducibility of these marks due to repeated firings.

This study examines the origin and production of dust cover impact marks on cartridge cases fired in a 7.62x39mm rifle; specifically, the changes that occur from extended or repeated use of the firearm. Two hundred brass- and steel-cased cartridges were fired using a new dust cover on an AK-type firearm. High-Speed Video (HSV) was utilized to confirm the specific location of impact on the dust cover and identify the differing categories of impact marks that resulted based on impact location. Changes to the morphology of the dust cover were observed and documented. It was determined that these changes occur over repeated firings, effecting and likely reducing microscopic comparison qualities. The degree of change to the dust cover is dependent on the type of ammunition used.

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**Dust Cover, AK-47, 7.62x39**

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**E95 An Open-Source Implementation of the Congruent Matching Profile Segments (CMPS) Algorithm for Assessing the Similarity of Bullets**

*Wangqian Ju, MSc\*, Iowa State University, Ames, IA; Heike Hofmann, PhD, Iowa State University, Ames, IA*

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**Learning Overview:** Attendees will be introduced to an open-source implementation of the CMPS algorithm; they will learn about a principled evaluation framework of algorithmic results, which allows a comparison and cross-validation of parameter choices. Attendees familiar with the statistical software R will learn how to use the CMPS algorithm with the R package CMPS.

**Impact Statement:** More and more areas of forensic science are incorporating results from algorithms in the evaluation of forensic evidence to make results objective and reproducible. Open-source algorithms are needed for a fair execution that is transparent and accessible to everybody.

The R package CMPS introduces an open-source implementation of the CMPS method developed at the National Institute of Standards and Technology (NIST) for objective comparison of striated tool marks.<sup>1</sup> The functionality of the package is showcased by examples of bullet signatures that come with the package. Graphing tools are implemented in the package as well for users to assess and understand the CMPS results. Initial tests were performed on bullet signatures generated from two sets of 3D scans in the Hamby study under the framework suggested by the R package bulletxtrctr.<sup>2-4</sup> New metrics based on CMPS scores are introduced and compared with existing metrics. A measure called Sum of Squares Ratio is included, and how it can be used for evaluating different scans, metrics, or parameters is showcased with the Hamby study data sets. An open-source implementation of the CMPS algorithm makes the algorithm more accessible, generates reproducible results, and facilitates further studies of the algorithm, such as methods comparison.

**Reference(s):**

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3. J.E. Hamby; D.J. Brundage; N.D.K. Petraco; J.W. Thorpe. A Worldwide Study of Bullets Fired From 10 Consecutively Rifled 9MM RUGER Pistol Barrels—Analysis of Examiner Error Rate. *Journal of Forensic Sciences*, 64(2):551–557, 2019. ISSN 1556-4029. doi: 10.1111/1556-4029.13916.
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**3D Topographical Measurement, Pattern Matching, Firearm Evidence**

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## E96 Blind Testing in Firearms: Preliminary Results From a Blind Quality Control Program

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**Learning Overview:** This presentation will inform attendees on preliminary findings from a blind testing program in the Firearms section at the Houston Forensic Science Center. Additionally, this presentation will detail trends in the data and discuss the benefits, limitations, and future directions of the blind testing program.

**Impact Statement:** The intent of this presentation is to evaluate a blind testing program in firearms examination and demonstrate how such a program can be a useful tool for assessing examiner and workflow processes. Attendees will be encouraged to consider the feasibility of incorporating similar blind testing programs into their laboratories' workflows.

Open proficiency tests created by external vendors meet accreditation requirements and help demonstrate examiner competence; however, the tests are limited in the ability to test the entire quality management system from evidence submission to reporting of results. Blind proficiency testing has been considered a more precise testing an individual's accuracy.<sup>1</sup> In December 2015, the Houston Forensic Science Center implemented a blind Quality Control (blind QC) program in firearms examination. The intent of the blind QC program is to supplement open proficiency tests by creating mock cases that mimic routine casework, so examiners are unaware that they are being tested. Blind QC cases are created by Firearms section management but submitted by members of the Quality Division, which is organizationally separate from the analytical sections.

This study examined the results of blind QCs submitted to the Firearms section for analysis. Firearms section management evaluates the created evidence prior to submission to determine the expected results and reviews the results of the completed blind QCs to determine satisfactory completion. A satisfactory result may include: (1) a result that conforms to the known ground truth, or (2) a result that does not necessarily conform to the known ground truth but is technically sound (i.e., a known elimination/identification that is reported as inconclusive based on the applicable standards in the field).<sup>2</sup> A firearms examiner can choose to conclude inconclusive if the item does not contain the quality or quantity of information needed to include or exclude from another item. Fragments, bullet cores, and poor-quality items are expected to be reported as unsuitable or insufficient regardless of ground truth based on availability of microscopic characteristics.

Fifty-one blind QC cases were reported between December 2015 and June 2021 resulting in 570 sufficiency and comparison conclusions. In 40.3% ( $n=225$ ) of the comparisons, the ground truth result was either elimination or identification, but the examiner made an inconclusive conclusion. Variables such as the examiners assigned to the case, the training program, the examiner experience level, and the intended complexity of the case did not significantly contribute to the inconclusive results. The main factor contributing to the inconclusive conclusions was the type of evidence compared (e.g., bullets vs. cartridge cases).

While a blind QC program can be challenging to implement, the program has demonstrated great value. The program demonstrates that the quality management system and procedures used by the Firearms section can obtain accurate and reliable results and provides examiners added confidence in court. Additionally, the blind QC program can be tailored to target specific research questions and provide opportunities for collaboration with other laboratories and researchers.

### Reference(s):

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2. Houston Forensic Science Center. Firearms Section SOP. <https://records.hfscdiscovery.org/Published/Firearms%20Section%20SOP%20Effective%20September%202011,%202020.pdf#search=8898>.

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### Blind Testing, Proficiency Testing, Firearms Examination

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**E97 Ordinal Regression for Error Rates in a Black-Box Face Recognition Study**

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**Learning Overview:** After attending this presentation, attendees will better understand: (1) how error rates in black box studies can be analyzed through ordinal regression method, and (2) how the uncertainty of these error rates can be quantified.

**Impact Statement:** This presentation will impact the forensic science community by introducing a regression method to evaluate ordinal decision scores from large-scale black-box studies, especially when error rates of multiple examiner groups are compared. The uncertainty quantification provides inherent sampling variabilities of the black box studies.

This study applied regression methods to assess error rates of face recognition based on a recent black box study by Phillips et al.<sup>1</sup> Participants included forensic facial examiners, facial reviewers, super recognizers, fingerprint examiners, and students. They were asked to provide ordinal-scale decision scores for image pairs based on their belief on whether the pairs belong to the same source. Scores are on a 7-point scale with +3 for the highest confidence of same source to -3 for the highest confidence of different sources. Image pairs from male and female were used in the study. Selected image pairs from Phillips et al. are shown in Figure 1.<sup>1</sup> Since human perception may exhibit differences regarding recognition of female faces and male faces, respectively, this study expected differences between the respective error rates. In addition, it is likely that the characteristics of source subjects may affect the error rates of face recognition.

The regression model includes the ordinal decision score as the response variable. The covariates in this dataset include race, gender, age of source subjects, and examiner groups. To accommodate ordinal decision scores, and in order to incorporate covariates of source subjects from the Phillips et al. black box study, the ordinal regression were employed instead of the regular linear regression.<sup>1</sup> The resulting covariate-specific receiver operating characteristic curves provide false positive rates and false negative rates. More importantly, the analysis provides the association between these error rates and characteristics of source subjects.

This study also calculated the error rates of examiner groups using the ordinal regression method and developed methods for calculating variances of these error rates to quantify the uncertainty for comparing examiner groups. Large-scale simulation studies were conducted to study the uncertainty of the error rates from simulated datasets. These simulation studies show that the error rates tend to have smaller variances as the sample sizes of black box studies increase.

**Reference(s):**

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**Facial Recognition, Error Rates, Uncertainty Quantification**

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## E98 A Forensic Decryption of Ludlings

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**Learning Overview:** Attendees can learn that there are forensic applications done by the Federal Bureau of Investigation (FBI) Lab that can deal with covert communication, up to and including criminals using ludlings like Pig Latin and Gibberish.

**Impact Statement:** The forensic science community will learn that there is an avenue to deal with unusual cases that cannot be typically dealt with outside of specific expertise, namely the ability to forensically decrypt covert communication.

Throughout time, criminals and law enforcement have undergone a never-ending battle to outwit each other. The eternal back and forth has resulted in the development of new scientific approaches and technologies that are astounding in their capabilities. However, the “old school” techniques should not be cast aside for the shiny and new.

The FBI Crime Laboratory has documented that criminals often use “old school” methods of cryptic communication to convey information about their crimes via phone calls from jails or prisons. One such method commonly encountered is the use of ludlings. Ludlings are seemingly simple language games that may even be heard on elementary school playgrounds. They are not true languages in and of themselves, but rather alterations of existing languages that are simple, yet effective, methods of concealing communications.<sup>1</sup> Potentially the most well-known ludling is Pig Latin. Pig Latin is not its own language. It is, at its core, the transposition and addition of English language syllables and can be translated by anyone familiar with the rules of the alteration scheme. However, there are many different versions and variations of ludlings, many with colorful names such as Ubbi Dubbi, Gibberish, Eggy Peggy, Tutnese, Ong, Sa-La, and many more.<sup>2</sup> Becoming so familiar with each ludling variation to be practically “fluent” is not a practical method of countering such covert communication. Therefore, the Cryptanalysis and Racketeering Records Unit at the FBI Laboratory has developed forensic standard operating methodologies to identify and decipher ludlings of any variation. Using these procedures, the FBI Laboratory has decrypted over 200 ludlings for law enforcement and testified in court proceedings, providing valuable evidence that has resulted in the identification of unknown accomplices, admissions of additional, yet unknown, felonies, the location of murder weapons, admissions of guilt, and much more.

**References):**

1. Phelan, R.; Simpson, D. 2020. Ludlings: Not all fun and games. *Cryptologia*.
2. Schwartz, A.; Zemach, M. 1982. *The cat's elbow and other secret languages*. New York, NY: Farrar, Strauss and Giroux.

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### FBI, Pig Latin, Ludlings

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## E99 Examining New Trends Exclusive to Latino Youth Gang Violence

*Cliff Akiyama, MPH, MA\*, Akiyama and Associates, LLC, Philadelphia, PA*

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**Learning Overview:** 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 recent Latino gang trends that could help law enforcement in their investigations of this ongoing deadly problem confronting all our communities.

**Impact Statement:** This presentation will impact the forensic science community by offering a detailed examination of novel Latino youth gang trends out on the street that have never been presented before to the forensic science community.

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 single state, meaning that gangs are no longer just a California problem. Nationwide, there are 26,500 gangs with a gang membership of over 850,000, while the ethnic composition of these gangs includes 47% Latino, 31% African American, 13% Caucasian, 7% Asian, and 2% Mixed. Youth gang violence in the Latino community has had a dramatic increase in the past two years since COVID-19. In Los Angeles County, CA, alone, there are currently 850 documented Latino gangs with a gang membership of over 120,000. Demographics show a gang member average age of 15 with a range of 8–22 years. 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 over 88% in some states. Over 800 gang members were interviewed out in the streets, jails, and juvenile halls in California and Pennsylvania. This study identified eight distinct manifestations and recent trends exclusive to Latino youth gangs 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; 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, they are run and controlled by one of two prison gangs, 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 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 is translated to mean “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.

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### Latino Gangs, Youth Violence, Law Enforcement

**E100 Exploring the Effectiveness of a Fingerprint Powder Batch Acceptance Protocol: Interdisciplinary Considerations**

*Helen Earwaker, PhD\*, University of Portsmouth, Portsmouth, Hampshire, United Kingdom; Jodie Coulston, PhD, University of Portsmouth, Portsmouth, Hampshire, United Kingdom; Paul Smith, PhD, University of Portsmouth, Portsmouth, Hampshire, United Kingdom*

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**WITHDRAWN**

## E101 Magnetic Nanoparticles Combined With Mass Spectrometry for the Extraction and Detection of Drugs and Toxic Metal From Fingermarks: A Novel Approach for the Forensic Chemist

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**Learning Overview:** The present work demonstrates the use of magnetic nanoparticles and Surface-Assisted Laser Desorption/Ionization-Mass Spectrometry (SALDI-MS) to extract and detect fingerprint components. This work will appeal to a broad audience, including analytical chemists in general and forensic scientists in particular. Attendees will learn the importance of combining magnetic material and MS to analyze various fingerprint and forensically relevant samples that may be recovered from the crime scene with minimum sample consumption and pretreatment. Furthermore, attendees will be exposed to a facile approach to fabricate magnetic material using by-product from burnt candles (soot) that can be employed in different applications. Moreover, forensic scientists will be interested in the recovery of the fingerprint components from different surfaces and understand the potential contribution of each surface using a simple magnetic field to maneuver the magnetic substrate. Finally, this study will shed more light on the (bio)chemical composition of fingerprints as a source of a vital forensic evidence.

**Impact Statement:** For decades, the analysis of fingerprints has been used as the primary biometric means of human identification because no two people share the same fingerprint. Modern chemical analysis technologies have added the potential to determine the molecular composition of fingerprints and identify chemicals that the suspect may have encountered. Improvements in analytical detection of the chemical composition of fingerprints is therefore of obvious importance. This presentation will offer a facile approach to synthesize magnetic carbon nanoparticles from candle soot for extraction and detection of endogenous and exogenous components of the fingerprint from different surfaces using SALDI-MS. Specifically, this work covers extraction of several exogenous compounds such as antihistamines, antidepressants, antibiotic drugs, and lead ions. Successful extraction and detection of these compounds may help to understand or solve cases related to death by gun, suicide or poison. This will stimulate researchers with various backgrounds and interests to contribute new ideas and implement novel approaches. In addition, forensic practitioners will learn about emerging technologies and commit to their testing, evaluation and further development. New developed technologies for fingerprint analysis could be helpful and potentially adapted in various other areas, including medical diagnostics and tissue analysis, in particular.

For decades, the analysis of Fingerprints (FP) has been used as the primary biometric means of human identification. In parallel, the chemical analysis of Latent Fingerprints (LFM), “touch chemistry,” offers additional support to forensic examinations; thus, the development in analytical detection of the FM is crucial. In this regard, this study proposes a facile approach to synthesize Magnetic Carbon Nanoparticles (MCNPs) from candle soot for extraction and detection of endogenous and exogenous components of the FM. In this context, endogenous components of FM, including Fatty Acids (FAs), squalene, and Triacylglycerols (TAGs), were successfully extracted and detected using SALDI-MS. The proposed method was used for personal identification based on profiling organic materials, including antihistamines (methapyrilene and triprolidine), antidepressants (metoprolol and labetalol), and antibiotic (metronidazole and sulfamethoxazole) drugs, in FMs. This was further emphasized using high-energy collision-induced dissociation, which provided characteristic and unique fragmentation patterns of each drug in the FM matrix. Furthermore, exogenous contamination of lead ion ( $Pb^{2+}$ ) on the suspect can aid in investigating cases related to death by gun; thus, this study has also successfully applied this approach for extracting inorganic toxic materials such as lead ions in FM with Limit Of Detection (LOD) in the nanorange. On the other hand, securing the crime scene and sample collection are vital steps for the evidence to be admissible in a court; therefore, the study reports a preliminary assessment of the influence of environmental conditions on the stability of the exogenous contaminants in FM. The contaminated FMs were exposed to different temperatures over 24 hours, and the results suggested that drugs were unstable at high temperatures and underwent different degrees of degradation, whereas lead ions were more stable toward thermal stress. Additional experiments were undertaken to investigate the presence of exogenous materials in FM upon handling drug tablets, and the findings indicated the presence of the drugs and/or their fragment in the spectrum as illustrated in Figure 1. The LOD values of the drugs in the tablet forms were 50, 200ng mL<sup>-1</sup> and 750ng mL<sup>-1</sup> for triprolidine, metoprolol, and sulfamethoxazole, respectively. Quantitative analysis of contact residues on fingers for parent drugs was estimated and recovery rates of 91.17, 94.67, and 120.86% were obtained for triprolidine, metoprolol, and sulfamethoxazole, respectively. Finally, a proof-concept study was constructed to extract and detect components of FM, following contact with traces of metoprolol commercial tablets, from metal and glass surfaces. Ultimately, the proposed method offered satisfactory extraction and detection efficiencies of FM components on both surfaces. Despite current FM analysis involved, several techniques, including Gas Chromatography/Mass Spectrometry (GC/MS), Raman spectroscopy and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS), combining magnetic material with SALDI-MS for extraction and detection of FM components reinforce the importance of the current method in forensic analysis. Thus, the present study provides exciting opportunities for the use of MCNPs as new SALDI-MS substrate for both extraction and detection of FM components, providing more information pertaining to the donor’s lifestyle.

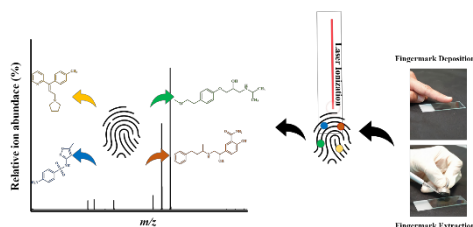


Figure 1: Schematic illustration of the experimental approach employed for the extraction and analysis of a fingerprint contaminated with drugs using SALDI-MS. Initially, the contaminated fingerprint was deposited on a surface and extracted using magnetic nanoparticles. Then, the nanoparticles were collected and deposited on the target plate for SALDI-MS analysis.

### Fingerprint, Mass Spectrometry, Drugs



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## **E102 The Use of Morphological Image Processing and MATLAB® Tools in Fingerprint Forgery—An Emerging Challenge**

*Awais Amin, MS\*, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan*

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**Learning Overview:** Attendees will learn advanced techniques of forgery while evaluating fingerprints on the documents. This presentation will enable experts to distinguish between original and forged fingerprints.

**Impact Statement:** This presentation will enlighten the forensic community about how new techniques are being used in documents forgery and it will enable them to counter these techniques and differentiate between an original fingerprint and a forged one.

Fingerprints have been used as an important tool in combating crime and identifying individuals for decades. However, challenges of fingerprint imitation and forgery were not predicted in the beginning. Forged fingerprints can sidetrack law enforcement agencies and forensic scientists during the process of incriminating criminals. Many unique and advanced techniques are available nowadays to forge fingerprints. Therefore, fingerprint experts need to be aware of all the forgery techniques so that undetected forged fingerprints may not contribute toward a false conviction or exoneration.

The use of rubber stamps is one of the widely used techniques to forge fingerprints. However, individualizing characteristics like manufacturing defects, impression voids, coincidental peripheral printing, absence of variation in boundaries and overlays—in case of multiple prints produced by same rubber stamp—are used to distinguish between forged and genuine fingerprints.

Advances in technology are available to both the scientific community and forgers. This research will enlighten the forensic community about the possible fraudulent use of morphological image processing and MATLAB® tools to remove any defects in the image of a genuine fingerprint for subsequent production of rubber stamps and the challenges posed to fingerprint experts to detect such kinds of forgeries.

In this research, genuine inked fingerprints from an original document were scanned and processed in the above-mentioned image processing tool to convert them into black and white images. Friction ridges were represented by black pixels. The images of fingerprints used in this study contained many defects and, if transferred to rubber stamps through laser technology, can easily be detected by a trained fingerprint expert. Morphological image processing was performed on these images using multiple tools available in MATLAB®. These operations included morphological erosion, dilation, opening and closing with multiple structuring elements like diamond- and square-shaped structuring elements. These image-processing operations sharpened friction ridges, removed small clumps of undesirable noise pixels, and eliminated small texture fluctuations while preserving the original shape and size of friction ridges. These processed fingerprint images were transformed to rubber stamps using laser technology. Fingerprints were carefully forged on the documents using these rubber stamps and, despite having exposure and training, fingerprint experts were unable to distinguish between the original and forged fingerprints.

It is therefore advised that fingerprint experts should consider the aspects of such advanced techniques when evaluating forgery of fingerprints. The natural pressure variations available in genuine fingerprints should be given more significance in such cases.

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**Fingerprints, Forgery, MATLAB®**

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## E103 Investigative Leads in Latent Prints: A Comparison of Laboratory Procedures

*Amanda Wilson, MS\*, King County Regional AFIS, Seattle, WA; Sharon Kelley, JD, PhD, University of Virginia, Charlottesville, VA; Jeniffer Molina, BS, Houston Forensic Science Center, Houston, TX; Brett Gardner, PhD, University of Virginia, Charlottesville, VA; Maddisen Neuman, MA, University of Virginia, Charlottesville, VA*

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**Learning Overview:** This presentation will educate attendees about how different latent print sections have defined “investigative leads.” This presentation will describe the procedures used by two different latent print sections to arrive at investigative leads and will discuss the relative strengths and limitations of each procedure.

**Impact Statement:** This presentation will encourage attendees to think in more critical and specific terms about a commonly used phrase. Attendees will also hear from examiners about relative strengths and weaknesses of different investigative lead procedures, allowing them to carefully consider the types of procedures that might be optimal to implement in their labs.

**Introduction:** Forensic science laboratories sometimes report information to law enforcement as “investigative leads.” Based on a review of available literature across several disciplines (e.g., latent prints, chemistry, biology, trace), the term investigative lead is generally used to refer to information that points to the possibility of an individual as the source of evidence relevant to a crime, but falls short of being a conclusive individualization or identification.<sup>1-3</sup>

Interestingly, although an investigative lead appears to be a well-understood concept in general, the specific procedures and thresholds different laboratories use in defining investigative lead can vary tremendously. In theory, these procedural differences could affect the reliability of the information reported out and the overall utility of the information to a criminal investigation. This presentation will describe and contrast two laboratories’ operational definitions of investigative lead and the associated procedures and thresholds used before information is reported to law enforcement.

For the past five years, the Latent Print Section of the Houston Forensic Science Center (HFSC) has reported investigative leads in the form of “Preliminary Automated Fingerprint Identification System (AFIS) Associations” (PAA). PAAs occurred when an analyst determined that there was sufficient agreement to conclude that two prints may have originated from the same source. PAAs do not involve the full set of procedures in the traditional Analysis, Comparison, Evaluation-Verification (ACE-V) process and instead involve a truncated process so that information can be reported out to law enforcement more efficiently. Importantly, investigative lead reports involve clear language about the nature of the comparison (e.g., “An official identification HAS NOT been effected at this time ...”). Submitting agencies can request official confirmatory comparisons if they choose. In sum, these procedures aim to balance accuracy and expediency so that law enforcement receive useful information earlier in the investigation than they otherwise might without such procedures.

The King County Sheriff’s Office AFIS Identification Section defines investigative leads quite differently. Investigative leads are reserved for AFIS comparisons that result in inconclusive conclusions (i.e., AFIS inconclusives) that are then reviewed using the lab’s “Consensus Judgment” procedures. At King County, “Consensus Judgment” requires a minimum of five examiners (including the original examiner) to independently examine, compare, and chart the impressions. The group must determine whether the amount of consistency between the latent and AFIS candidate is “marginal” or “considerable;” comparisons with considerable consistency are reported as investigative leads. The unit supervisor then reviews all documentation to determine if the consensus decision complies with the standard operating procedures or if additional testing is warranted. Thus, these procedures utilize more extensive review (rather than more expedient review) to determine whether inconclusive information is sufficiently reliable to be of use in a criminal investigation.

Overall, these two sets of procedures highlight the strikingly different ways that latent print sections can define investigative leads: preliminary identifications versus extensively reviewed inconclusives. This presentation will explain these procedures in more detail, include case examples, and discuss their relative strengths and limitations, as perceived by examiners within each laboratory.

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1. Attias, D.; I. Hefetz; E. Ben-Shimon. Latent fingerprints of insufficient value can be used as an investigative lead. *Journal of Forensic Science and Criminology* 3 (2015): 1-4.
2. Lavine, B.K.; A. Fasasi; M. Sandercock. Improving PDQ database search strategies to enhance investigative lead information for automotive paints. *Microchemical Journal* 117 (2014): 133-137.
3. Rashaid, A.H.B.; P.B. Harrington; G.P. Jackson. Amino acid composition of human scalp hair as a biometric classifier and investigative lead. *Analytical Methods* 5 (2015): 1707-1718.

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### Latent Print Comparison, ACE-V, Investigative Lead

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## E104 Measuring Proficiency Among Latent Print Examiners: A Statistical Approach From Standardized Testing

*Amanda Luby, PhD\*, Swarthmore College, Swarthmore, PA*

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**Learning Overview:** After attending this presentation, attendees will have learned about a statistical approach to quantifying the proficiency of latent print examiners used prominently in standardized testing.

**Impact Statement:** This presentation will impact the forensic science community by offering a statistical method for evaluating the performance of latent print examiners in making source decisions. The statistical framework introduced is flexible and easily adaptable to many different decision-making scenarios.

In recent years, the reliability and validity of forensic analysis has received increased attention in many pattern evidence disciplines in forensic science. The current approach to characterizing uncertainty and variability in these disciplines has largely centered around conducting large-scale error rate and “black box” studies in order to calculate aggregated error rates.

The Federal Bureau of Investigation (FBI) “Black Box” Study is one such study that was designed to estimate casework error rates of latent print comparisons in the United States.<sup>1</sup> This study provided critical and necessary information about overall performance aggregated over many comparisons and set the bar for conducting “black box” studies in other pattern evidence disciplines. However, we cannot directly compare individual examiners’ error rates, since each participant was asked to evaluate a random subset of comparison tasks (items) and some items are more difficult than others due to, for example, partial prints or low image quality.

Item Response Theory (IRT), a class of statistical methods used prominently in educational testing, can be used to measure participant proficiency in error rate studies while simultaneously accounting for varying difficulty among items.<sup>2</sup> Using data from the aforementioned “Black Box” study, this presentation will explore the strengths of an IRT-based analysis over traditional “percent correct” scoring. Challenges in wide-scale implementation will also be discussed.

Using an IRT-based analysis, the largest variability in latent print examination decisions tends to occur in print quality assessments and inconclusive decisions, which is consistent with prior work.<sup>3</sup> Some participants were likely to over- or under-report difficulty even after accounting for their proficiency, item difficulty, and other participants’ reported difficulty; and examiners who report items to be more difficult perform similarly to examiners who report items to be easier. These results underscore the importance of better understanding the cognitive factors involved in latent print examination decisions.

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1. Ulery, B.T.; Hicklin, R.A.; Buscaglia, J.; Roberts, M.A. (2011). Accuracy and reliability of forensic latent fingerprint decisions. *Proceedings of the National Academy of Sciences*, 108(19), 7733-7738.
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### Latent Print Analysis, Statistics, Human Factors

**E105 Modeling Latent Fingerprint Aging Processes by 2D Optical Methodologies**

Josep De Alcaraz-Fossoul, PhD\*, University of New Haven, West Haven, CT

**Learning Overview:** In this presentation, attendees will learn about modeling latent fingerprints' age estimation in 2D by using a digital camera and other common techniques. The model involves logistic regressions based on binning fingerprint data by age, which includes quantitative and qualitative measurements and assessments. The combined use of four morphometric-based age indicators is the foundation for time of deposition estimations.

**Impact Statement:** This presentation will impact the scientific community by demonstrating the feasibility of fingerprint age estimations that are based on measuring 2D topographical changes acquired by optical means alone in the visible light spectrum. This is a significant step forward to further strengthen the probative value of fingerprints and reduce potential bias in identifications.

For more than a century, fingerprinting methodologies have relied primarily on friction ridge skin patterns for the identification of crime suspects and victims.<sup>1</sup> While questions related to the source (the “who”) have already been the topic of most research and publications, the issues associated to the activity-level (the “when”) have only begun to be explored in depth more recently.<sup>2</sup> Despite decades of research and frequent court enquiries, the estimation of age by simple optical means is still perceived, in legal proceedings, as unreliable and subjective, frequently founded on the personal opinion of the forensic scientist, and often disqualified as reliable evidence.<sup>3-6</sup>

Surprisingly to this day, there are no mathematical models or computer algorithms to estimate the age of latent fingerprints (i.e., time elapsed from deposition to discovery). This type of evidence is invisible to the naked eye and requires enhancement treatments for visualization prior to analysis. Alterations of a fingerprint's topography as it ages have been described for years.<sup>3,7</sup> It has been proven that some of these physical changes are time-sensitive, including modifications in ridge width and loss in color contrast once powdered in response to the surrounding environment (indoors).<sup>8,9</sup> These are potential sources of data that can be used to determine the age of the impression; especially on non-porous surfaces.

In this research, four morphometric-based indicators of aging have been evaluated and combined to create the first mathematical model to estimate the time of deposition of powder-enhanced latent fingerprints. Experiments were designed to closely mimic field conditions, from deposition to visualization and analysis. It combined a pool of 34 different donors over a period of ten years, totaling 2,218 fingerprints photographed with a digital camera. Fingerprints were aged for up to 364 days while exposed to a variety of indoor conditions and enhanced with titanium dioxide or carbon black powders. Non-porous substrates (plastic, glass, or tile) and two sweat secretion types (sebaceous or eccrine-rich) were exposed to three natural light conditions—direct light, shade, or darkness. These images were then evaluated by ten examiners on the following visual indicators: (1) Quantitatively: loss of viable minutiae, differences in color contrast between ridges and furrows, presence of ridge discontinuities, and micro-variations in ridge width; and (2) Qualitatively: collection of ridge pattern Quality Scores (QS) on a scale from 1 to 4 and/or color-coded maps by the Universal Latent Workstation.<sup>8-14</sup>

In this project, the most influencing environmental factors involved in aging have been identified; for example, contrary to common belief, exposure to natural light does not necessarily accelerate degradation, while the type of substrate is the most significant factor of all those examined, followed by secretion type. In addition, biological sex, age, ethnicity, dietary regimens, and ridge type pattern show no distinctive impact on aging. Most importantly, the mathematical formulation allows for the categorization of age into: fresh, intermediate, and advanced degradation; while more research is needed for actual age estimations and error rates.

This proof-of-concept research concedes certain necessary limitations, including that glass substrates do not show significant ridge degradation under certain conditions; the use of specific powders for visualization; and the minimum quality threshold necessary for the analysis. Despite these constraints, the practical implications of these findings are enormous and an important step forward for forensic science. The results challenge the conventional assumption that external influences are too complicating a factor. In conclusion, this study has been able to successfully demonstrate and model with a high degree of accuracy the dynamics of the aging complexity of fingerprints under semi-controlled situations, introducing new kinetics of aging.

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**Aging, Morphometrics, Fingermark**

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## E106 Similarities of Fingerprints Among Siblings

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how powerful fingerprint impressions are for personal identity. This is a comparative study aimed at determining if siblings have more similar fingerprint pattern characteristics than people not related.

**Impact Statement:** This presentation will impact the scientific community by looking at the following patterns and subtypes: arches (plain and tented), loops (left slant, right slant, whorls), plain, center pocket, double loop, and accidental. The objective of this study is not to refute any of the previous research on twins, rather it is to determine whether siblings have a higher rate of matching pattern types than non-siblings.

Over the past hundred years, research has shown that no two individuals have the same fingerprint. The ridge arrangement, placement, ridge size, and minutiae within the pattern are unique to the individual. These are evaluated as Level 1, Level 2, and Level 3 characteristics as determined by latent print examiners. Fingerprints offer an irrefutable means of personal identification. That is the underlying basis for establishing the identities of criminals. It is also used as a means of identification for missing persons, disaster victims, and unidentified human remains. Other uses are for personal identification for employment, to obtain applications for driver's licenses, and security entrance to government buildings. Other individual characteristics change, especially over time (with the exception being DNA); fingerprints can distinguish identical twins

There are 11 sets of biological siblings, including 1 pair of identical twins and 22 unrelated individuals that did not have a biological sibling in the study. The 11 sibling pairs have the same biological mother and father. The subject pools consisted of both genders (males, females) and are aged from 13 to 75 years.

A total of 44 fingerprints were evaluated by a latent print examiner for Level 1 ridge flow patterns (Loops [L], Whorls [W], and Arches [A]) and for their orientation. Fingerprints were obtained by rolling each of the ten fingers on an ink-pad and then pressing each inked finger on a ten-print file card.

A 5x "loupe" magnifying glass was used to examine each fingerprint pattern from the ten fingers of siblings and unrelated individuals. The data was entered into an EXCEL<sup>®</sup> spreadsheet. Analysis of Variance (ANOVA) and chi-square statistical tests were used to determine if there were significant differences (or not) in the totals of the fingerprint patterns. Significance in all statistical tests determined when p-values <.05. Spearman correlation test was done to see if any significant correlation existed between the observed patterns in the ten fingers: L vs. W, W vs. A, and A vs. L patterns in the sibling (and unrelated individual) data. A good correlation was considered when correlation coefficient  $R_s = \pm 0.6$ . Lastly, the number of shared characteristics whether L, W, or A between sibling pairs was compared to randomly paired, unrelated individual fingerprints. This data was analyzed by the Mann-Whitney test.

ANOVA indicated that totals of fingerprint pattern types were significantly different in hands of the siblings (ten fingers) and in the unrelated individuals ( $L > W > A$ ; p-value <.0001). In fact, the number of Loops was over twice the number of W and A combined. Chi-square test results indicated that there was no significant difference (p-value >.05) between these numbers for the siblings and unrelated individual fingerprints. There was also a significant inverse correlation ( $R_s = -0.6$ ) between L vs. W, W vs. A patterns (p<.01), but no significant relationship between Loops and Arch patterns. It was thought that siblings would have more similar pattern types than unrelated individuals. However, there was no difference in the number of similar pattern types in the pair studies between siblings and unrelated individuals (p value=.07, mean 5.45 vs. 4.19).

Fingerprint patterns were significantly different in siblings (not unexpectedly). This was also found in unrelated fingerprint pattern types. There were more Loop patterns than Whorl and Arches combined. There was no difference in distribution of fingerprint pattern types between siblings and from unrelated individuals. This study indicated there was a significant inverse correlation between L and W and W and A patterns. There was no significant relationship between L and A patterns. The use of LiveScan technology will supplant other methods establishing identities of criminals.

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### Sibling Fingerprints, Criminal Investigations, Fingerprint Patterns

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## E107 An Examination of Pattern Types in Twins' Fingerprints and Bilateral Symmetry in an Unrelated Population

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**Learning Overview:** After attending this presentation, attendees will have a better understanding regarding if there are stronger genetic or environmental factors affecting fingerprint pattern development or another influence during fetal growth. The objective of this research is to indirectly assess the genotype versus environment influence on phenotype variation of fingerprint pattern types of monozygotic twins and dizygotic twins. This study examined the percentage of intra-pair variance of the monozygotic twins and dizygotic twins fingerprint pattern types homolaterally and bilaterally for their symmetry and compare their frequency statistics to a large population. In a separate study of 300 individuals regarding fingerprint pattern bilateral symmetry, 3,000 rolled fingerprints were examined and classified for each finger, 1-10, and opposing fingers were compared.

**Impact Statement:** This presentation will impact the forensic community by providing a more in-depth understanding of symmetry in fingerprint pattern types in twins both bilaterally and homolaterally and their pattern frequency statistics. Additionally, this study presents the frequency of bilateral symmetry of the right and left hands in an unrelated population.

Many researchers have investigated whether it is our genetics (genotype) that drive the development of pattern types or the environment, maternal effects in the mother's womb, that have a stronger influence on fetal pattern formation. Researchers consider it as a combination of our genotype and the environment that creates a phenotype using this formula:  $VP=VG+VE$ . A sum of 222 monozygotic twins and 56 dizygotic twins' fingerprints were collected for a classical twin study in dermatoglyphics to better understand the genetic and environmental influences in fingerprint pattern formation.

A total of 2,215 monozygotic twins' fingerprints and 560 dizygotic twins' fingerprints were examined and classified using the eight classifications: plain arch, tented arch, ulnar loop, radial loop, double loop whorl, plain whorl, central pocket loop whorl, and accidental whorl. Using the National Crime Information Center (NCIC) fingerprint classification codes, each finger's pattern was recorded, then compared homolaterally and bilaterally. In a separate study, a total of 3,000 rolled fingerprints were examined and classified using the same eight classifications. Using the NCIC codes, each finger's pattern was recorded, then compared bilaterally.

The homolateral concordant pattern types for the monozygotic twins' fingerprints (70.9%) exceeded the frequency of the dizygotic twins' fingerprints (58.1%). The frequency of bilateral symmetry in the monozygotic twins was 63.2%, less than the homolateral symmetry between the monozygotic twins 70.9%. The frequency of bilateral symmetry in the dizygotic twins was 49.6%, less than the homolateral symmetry between the dizygotic twins 58.1%. Seemingly, monozygotic and dizygotic twins' hands have more symmetry homolaterally to their twin than bilaterally on their own hands. The chi-square test found with both groups was a statistically significant,  $p$  value  $<.0001$ . The frequency statistics of the twins' eight fingerprint classifications was concordant to those reported by NIST from 22,264,713 males and females. In the study of bilateral symmetry of fingerprint patterns in 300 unrelated individuals, bilateral symmetry averaged 69.8% with the same classification occurring 1,047 out of 1,500 paired fingers from the right and left hands. The chi square found it statistically significant,  $p$  value  $<.0001$ .

Fingerprint pattern formation is influenced by a third factor, in addition to genetics and environment: developmental noise. Developmental noise or randomness produced at the cellular level on the volar surface of fetus's fingers during the critical stage of fingerprint development affects ridge flow and thus supports the unique formation of friction ridge skin at the class characteristic level of pattern type. The updated formula for total variance of this phenotype is  $VP=VG+VE+VDN$ .

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### Dermatoglyphics, Twins, Fingerprints

## E108 An Analysis and Discrimination of Fingermarks Contaminated With Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Tablets Using Raman Spectroscopy and Chemometrics

Mohamed O. Amin, MSc\*, Kuwait University, Asimah, Kuwait; Entesar Al-Hetlani, PhD, Kuwait University, Asimah, Kuwait; Igor K. Lednev, PhD, University at Albany, Albany, NY

**Learning Overview:** The present work demonstrates the use of Raman spectroscopy as a non-destructive technique along with multivariate statistical analysis to detect traces of NSAIDs in Fingermarks (FMs) and determine the type of drug present. The work will appeal to a broad audience including analytical chemists in general and forensic scientists in particular, and attendees will recognize the potential of Raman spectroscopy to analyze various fingerprint and forensically relevant samples that may be recovered from the crime scene, suspect, or victim. Furthermore, the application of chemometrics, including multivariate Partial Least Squares Discriminant Analysis (PLSDS) and a Genetic Algorithm (GA) to differentiate between several FM samples, will emphasize the significance of these methods to gain a profound understanding of the results and differentiate between different forensic evidence.

**Impact Statement:** For decades, the analysis of fingerprints has been used as the primary biometric means of human identification because no two people share the same fingerprint. Modern chemical analysis technologies have added the potential to determine the molecular composition of FMs and identify chemicals that the suspect may have encountered. Improvements in analytical detection of the chemical composition of FMs is therefore of obvious importance. This presentation offers a novel approach to differentiate between NSAIDs-contaminated FMs. Raman spectroscopy with advanced statistic enabled the detection of traces of NSAIDs in FM and determining the type of drug present in the FM, which can be of profound importance in forensic intelligence. This will stimulate researchers with various backgrounds and interests to contribute new ideas and implement novel approaches. In addition, forensic practitioners will learn about emerging technologies and commit to their testing, evaluation, and further development. New developed technologies for FM analysis could be helpful and potentially adapted in various other areas, including medical diagnostics and tissue analysis in particular.

Over the past years, chemical analysis of Latent Fingermarks (LFMs), “touch chemistry,” has offered additional support to forensic examination and advanced our understanding of the individual’s lifestyle. Modern chemical analysis technologies have greatly contributed to the chemical analysis of the FM and identify materials that a suspect may have come in contact with. On the other hand, more than 30 million people take Over-The-Counter (OTC) and prescription NSAIDs for pain relief, headaches, and arthritis every day, which increases the risk of their abuse; in addition, NSAIDs have been commonly linked to suicide cases. The present study demonstrates the potential of Raman spectroscopy as a rapid and non-destructive technique along with statistical analysis for discriminating if FMs made contact with several NSAID tablets without any sample pretreatment (Figure 1).

Multiple Raman spectra were obtained from each FM sample using automatic mapping to account for the inherent heterogeneity of the sample. Features corresponding to the drugs were clearly observed in the Raman spectra in addition to several peaks that can be attributed to the excipients present in the tablets. Furthermore, chemometrics were used to determine whether Raman spectroscopic analysis can be used to distinguish between FM samples. Initially, a Principal Component Analysis (PCA) model was applied to identify outliers, defined as spectra with high Hotelling’s  $T^2$  and Q residuals values in all the data sets, and they were removed before any further statistical analysis. Multivariate PLSDS and GA were employed to differentiate between FM- and NSAID-contaminated FM groups with emphasis on the validation test to assure the applicability of the built models. A subject-wise Leave-One-Out Cross Validation (LOOCV) was utilized to examine the prediction performance of the models. The created PLSDA model using a training data set enabled the separation of sebaceous FM, aspirin-, diclofenac-, ibuprofen-, ketoprofen-, and naproxen-contaminated FMs, while LOOCV offered excellent results demonstrating the potential of the developed method for discriminating NSAID-contaminated FMs. Thus, the developed strategy is a promising approach to detect traces of NSAIDs and potentially other drugs in FM and determine the type of drug present, which can be of profound importance in forensic intelligence.

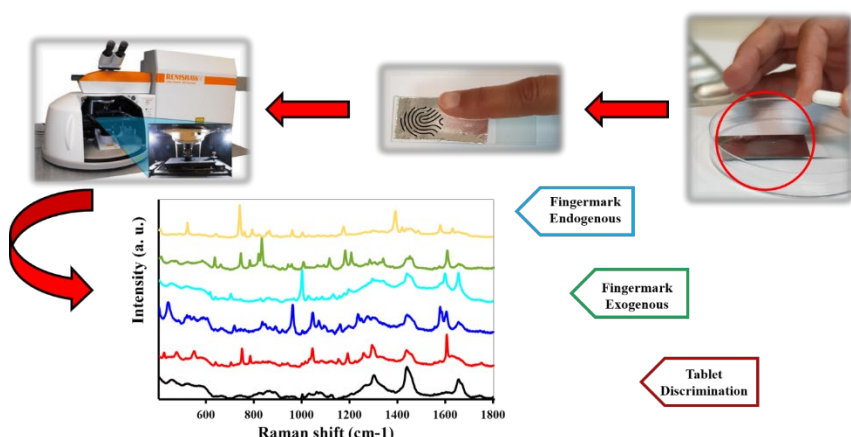


Figure 1: Schematic illustration of the experimental approach employed for the analysis FM following contact with NSAID tablets using Raman spectroscopy. Initially, the fingertip made contact with NSAID tablets, then the contaminated FMs were applied on the aluminum adhesive tape. The samples were subjected to Raman analysis and the averaged Raman spectra were obtained, which enabled the identification of FM endogenous and exogenous components in addition to the discrimination between commercial NSAID tablets with the aid of chemometrics.

### Fingermark, Raman Spectroscopy, NSAIDs

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**E109 The Terminal Deposition of Latent Fingermarks Determined by Chemical and Physical Developers**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of detecting the terminal latent fingerprint in a depletion series and the feasibility (i.e., detection limit) of different developers.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the level of efficacy of different developers at visualizing fingerprints in sequential depositions and the sensitivity to the loss of ridge quality between depositions using statistical data.

It is a known notion, through decades of field observations and experiments, that a finger ridge skin impression (i.e., latent fingerprint) loses its visual quality, or is no longer detectable, after repeated contact with a surface. However, what is unknown is the number of touches required until the impression goes beyond recognition for identification purposes. Being able to determine the terminal latent fingerprint and the gradual or sudden loss of ridge quality over the series could lead to new approaches in the visualization of fingerprints and different analytical techniques. The goal of this study is to establish the “terminal” deposition among a limited number of donors; that is, the point at which fingerprints can no longer be successfully visualized after a series of successive depositions without adequate time to recharge sweat residue on skin.<sup>1</sup> The additional goal is to determine and quantify the decrease in ridge quality between every deposition.

For decades, researchers have been studying and optimizing new methods for latent fingerprint enhancements.<sup>2</sup> However, previous studies have minimally touched upon the evaluation of the visual quality of fingerprint depletions, but they did not cover the level of loss of quality across deposition series. In fact, a lot remains unknown about how much ridge quality is lost as well as the overall progression of a series.

Physical developers, such as black magnetic powder, are commonly used to bind to the latent fingerprint residue, resulting in an enhanced color contrast between ridges and furrows that can be viewed by the naked eye.<sup>3,4</sup> Another way to visualize fingerprints would involve the application of a chemical developer such as cyanoacrylate (also referred to as super glue) to chemically react with the compounds contained within the latent fingerprint.<sup>5</sup> The comparison of the two techniques may establish which one is the most sensitive at detecting different levels of fingerprint depletion and yield the most accurate results on ridge quality.

The experiment involved the deposition of sebaceous-rich latent fingerprints in sequential series from four donors. These impressions were deposited on glass and visualized 24 hours after. All samples were standardized with Photoshop® and the quality of the ridges was analyzed through Universal Latent Workstation (ULW). ULW is a computer program that automatically provides a value for the clarity of ridges, among other information. Statistical programs such as Microsoft® Excel® and Statistical Package for the Social Sciences (SPSS) tests were used to record data and conduct statistical analyses.

Through the visual observation of the loss of ridge quality in a depletion series, detection of any inconsistencies across depositions of the donors was analyzed. Preliminary data suggests a significant loss of quality after the 14<sup>th</sup> and 15<sup>th</sup> deposition, proving that fingerprints could be detectable for many touches without needing to “recharge.” Visual observations indicated that cyanoacrylate may be too efficient for the purpose of depletion analyses, whereas black magnetic powder appeared to be more suitable at detecting the loss of ridge quality.

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**Fingermarks, Deposition, Sequential**

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## E110 The Development of Fingerprints After Prolonged Submersion in Freshwater

Haley Bolt, MS\*, Marshall University, Huntington, WV; Katrina Willis, Banks County Sheriff's Office, Homer, GA; Bruce Willis, Piedmont University, Demorest, GA; Catherine Rushton, Marshall University, Huntington, WV

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**Learning Overview:** After attending this presentation, attendees will have a greater knowledge of the development of fingerprints that have been submerged in freshwater sources for prolonged periods of time and the appropriate methodology to use.

**Impact Statement:** This presentation will impact the forensic science community by presenting experimental methods of two fingerprint development techniques that can be used on evidentiary items from the field.

A crucial aspect of crime scene investigations is the discovery and development of sufficient fingerprint evidence that can link a potential suspect or victim to a crime. Water scenes are somewhat unmarked territory when it comes to latent prints and their success rates. Several studies have been conducted previously on development techniques that could assist investigators in developing crucial prints after they had been submerged in water for extended periods of time, either because of delayed discovery of a scene or lengthy scene investigations.<sup>1-3</sup>

In 2017, one study was conducted by Somaya Madkour and her fellow associates on using cyanoacrylate fuming to develop prints on non-porous surfaces in fresh and seawater environments.<sup>2</sup> Their conclusions were that this development process was possible and worked efficiently with either water source; however the quality of the results decreased over time and the high salinity in saltwater damaged some of the prints.

The goal of this research study is to determine if cyanoacrylate fuming with a Rhodamine 6G Ardox M.B.D. dye stain (RAM) and Sudan Black are successful techniques for developing latent prints after being submerged in freshwater, and which method is more successful. The substrate types for the experiment will be non-porous and fall into one of three categories: aluminum, plastic, or glass. A trial study was conducted prior to an in-depth study to: (1) determine if either technique was even possible to use on these substrates, (2) eliminate any variables that would drastically affect the data, and (3) highlight which water type presented more information to expand upon.

In conclusion, the results indicated that cyanoacrylate fuming with RAM dye staining and Sudan Black were both highly responsive, with Sudan Black being more successful for sufficient print development. If any evidence was to be recovered from water scenes, regardless of the amount of time spent underwater, they should not be undervalued and should be tested for fingerprints using either of these methods.

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### Sudan Black, Fingerprint Development, Cyanoacrolate Fuming

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## **E111 Environmental Influences on Latent Fingerprint Recovery**

*Madison Frese, BA\*, University of Central Oklahoma, Edmond, OK; Caitlin Porterfield, MS, University of Central Oklahoma, Edmond, OK*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how temperature and humidity affect latent print stability and development over time.

**Impact Statement:** This presentation will impact the forensic science community by showing how environmental factors, specifically temperature and humidity, affect fingerprint recovery in outdoor crime scenes.

The goal of this study was to see if there are any noticeable differences in recovered fingerprint quality over time when prints are kept in outdoor environments. Specifically, this study will focus on how temperature and humidity impact latent print processing and development.

Because of the variability of environmental conditions, it is difficult to control for all factors. In an effort to account for factors outside of temperature and humidity, this study was conducted in two phases. Phase one involved developing prints over time in different locations around the United States in outdoor environments. This allowed for the assessment of fingerprint stability and quality at varying temperatures and humidity over time but accounted for other environmental factors. Lab simulations were also conducted to replicate these locations and conditions in an effort to determine the actual impact of the temperature and heat variables without the presence of other factors. Preliminary results indicate that temperature and humidity do impact the ability to recover prints.

A difficult part of fingerprint analysis is figuring out how long a fingerprint has been at a scene. So far, there is no verifiable method for determining age.<sup>1</sup> If it is found that there is a pattern in recoverability and quality using temperature and humidity, this may allow for the creation of a method for determining relative age for outdoor crime scenes in different environmental conditions.

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

- <sup>1</sup> Girod, A.; Ramotowski, R.; Lambrechts, S.; Misriellal, P.; Aalders, M.; Weyermann, C. (2016). Fingerprint age determinations: Legal considerations, review of the literature and practical propositions. *Forensic Science International*, 262, 212–226. <https://doi.org/10.1016/j.forsciint.2016.03.021>.

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### **Fingerprint Analysis, Environmental Conditions, Recovery of Fingerprints**

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## E112 Forensic Phenomics—An Applied Approach to Fingerprint Profiling for Intelligence Guided Investigations

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**Learning Overview:** Upon the conclusion of this presentation, attendees will understand the current analytical techniques and future direction for the application of phenomics to forensic fingerprint evidence in the investigative landscape.

**Impact Statement:** This presentation will impact the forensic community by raising awareness of the field of forensic phenomics and its potential applications in criminal investigation and national security.

The identification of a perpetrator of crime remains the foremost significant aspect of forensic investigations; however, as forensic awareness increases within the community, the nature of evidence left behind at the crime scene has begun to change. As a result, the identification of new methods and techniques for the identification of an individual have become increasingly important.

To overcome current limitations imposed by fingerprint examination and comparison techniques for identification—such as the Automated Fingerprint Identification System (AFIS)—this study focused on the examination and exploitation of the chemical composition of fingerprint residue, including endogenous, semi-exogenous, and exogenous compounds present in the residue.<sup>1</sup>

This study analyzed repeated fingerprint samples from 20 participants using a combination of mass spectrometric techniques, including mass spectrometry imaging using Matrix-Assisted Laser Desorption Ionization (MALDI) and Desorption Electrospray Ionization (DESI); and targeted liquid extractions of fingerprint residue swabs using Liquid Chromatography/Mass Spectrometry (LC/MS).

Identification of observed components was conducted using a combination of publicly available databases with statistical evaluation carried out to identify data trends and prominent unique features able to distinguish individuals, highlighting the applicability of the approach.

By increasing the tools available to those involved in criminal and national security investigations, it may be possible to identify points of investigative intelligence that can help identify suspects, help establish the order of events, or confirm/refute eyewitness statements concerning criminal activities. This research provides evidence for MS-based analytical techniques in forensic profiling and intelligence gathering during criminal and national security investigations and highlights key focus areas for the expansion of forensic phenomic research.

**Reference(s):**

- <sup>1</sup> Girod, A.; Ramotowski, R.; Weyermann, C. Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci Int.* 2012;223(1–3):10–24.

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### Phenomics, Fingerprint Profiling, Mass Spectrometry

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## **E113 The Impact of Packaging and Transportation on the Preservation and Processing of Latent Friction Ridge Impressions**

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**Learning Overview:** After attending this presentation, attendees will understand the impact that packaging and transportation of items of evidence have on the ability to preserve and process latent friction ridge impressions deposited on those items.

**Impact Statement:** This presentation will impact the forensic science community by establishing the best method for preserving and processing latent friction ridge impressions. It will provide insight into whether it is better to process items at the scene or transport them to the lab. It will also indicate how packaging and length of transportation impact latent friction ridge impressions.

This study was conducted to determine if packaging and transportation of small items of evidence impacts the ability to preserve and process latent friction ridge impressions deposited on those items. Similar research has been completed with DNA evidence and researchers found that packaging and transportation did impact the quality of the DNA evidence when it was analyzed.<sup>1</sup> Since it has been established that packaging and transportation of items of DNA evidence can cause degradation, experts in the field are now working toward establishing best practices to reduce the level of degradation and improve the chances of recovering useable evidence.

This study focused on best methods for preserving and processing latent friction ridge impressions. Plastic bottles, aluminum cans, and ceramic white tiles were used as items of evidence. Fingerprints were deposited on the items and left for two hours to represent time between the crime being committed and the same crime being investigated. The evidence was then divided into two groups: one group processed without being transported and one group processed after being packaged in paper bags and driven in the car. These two groups represented evidence processed on scene and evidence processed after being packaged and taken to the lab. Time of transport ranged from 15 minutes to one hour. Temperature and humidity were also recorded. Latent impressions were then processed with black powder and the prints lifted with tape. Latent impressions were analyzed for level 1 detail (cores, deltas, pattern type) and level 2 detail (ending ridges, bifurcations, dots, etc.). The value of the friction ridge detail was then compared between the items processed at scene and those items of evidence that were transported. Time of transportation was also taken into consideration. Comparisons are currently being conducted. Preliminary results suggest that packaging and length of transportation do impact the stability and subsequently the processing of latent impressions.

The goal of this study was to establish best practices and guidelines for preserving and processing latent friction ridge impressions in an effort to maintain the highest possible level of quality and quantity of detail and provide more value during the analysis phase.

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

- <sup>1</sup> Goray, M.; van Oorschot, R.A.H.; Mitchell, J.R. (2012). DNA transfer within forensic exhibit packaging. Potential for DNA loss and relocation. *Forensic Science International: Genetics*, 6(2), 158-166.

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### **Fingerprint Processing, Fingerprint Analysis, Crime Scene Investigation**

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## E114 Raman Spectroscopy to Tackle the Analysis of Bloodstains in Crime Scene Conditions

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the impact of environmental conditions on determining the time since deposition of bloodstains.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of the broadened usability of Raman spectroscopy in forensic laboratories.

Blood traces are one of the most common biological samples found at crime scenes and can provide substantial information about individuals involved and the event that occurred. Blood evidence is frequently used for DNA analysis in order to create a genetic profile. However, currently the analysis of bloodstains is not being utilized to its full potential. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the Time Since Deposition (TSD) of bloodstains.<sup>1-3</sup> Additionally, if crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime and, therefore, reduce the number of samples that should be collected, documented, and processed.

Vibrational spectroscopy paired with chemometrics has been shown to provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains.<sup>4</sup> However, research conducted with these techniques so far has analyzed the aging of bloodstains, specifically the degradation of hemoglobin, in ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that is capable of estimating the TSD of bloodstains in different environments.

There are infinite varieties of potential environmental conditions. The goal is to determine how potentially “extreme” conditions affect the aging mechanism of bloodstains, high temperature in particular. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the *ex vivo* aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post-deposition. After the spectra were collected, they were loaded into statistical software for preprocessing and modeling. The reproducibility of heated blood analysis and a TSD determination model will be discussed.

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### Raman Spectroscopy, Time Since Deposition, Blood Analysis

## E115 Optimizing Blood Removal Methodology for Visualizing Gunshot Residue (GSR) Patterns

Ashley Smith, BS\*, Pennsylvania State University, University Park, PA; Michael Kuslusi, MFS, Pennsylvania State University, University Park, PA

**Learning Overview:** Through this presentation, attendees will gain a better understanding of photonic and chemographic visualization methods for GSR patterns, as well as the presence and removal of blood interferences.

**Impact Statement:** This presentation will provide more insight into photonic visualization of GSR patterns as a replacement for traditional chemographic processing. The impact of heavy bloodstaining on visualization and an evaluation of blood removal methods are also discussed.

GSR is the vapor and particulate matter that exits a firearm upon discharge, including smoke/soot, unburned and partially burned propellant, as well as components from the cartridge primer.<sup>1</sup> Visualization of GSR patterns is essential for shooting-incident reconstruction. Visualization of GSR patterns allows the muzzle-to-target distance to be estimated, based on the density and distribution of the residues on fabrics, skin, and other materials.<sup>1</sup>

Chemographic techniques such as the Modified Griess Test (MGT) and the Sodium Rhodizonate Test (SRT) are well characterized and have long been the methods of choice in forensic laboratories. However, these methods have practical limitations as well. In addition to potentially exposing examiners to chemical hazards, these methods are also destructive to GSR patterns.<sup>2</sup> This study is part of a larger effort to develop photonic techniques, such as blue-orange fluorescence and infrared reflectance, for the non-destructive visualization of GSR patterns.<sup>2,3</sup>

The presence of heavy bloodstaining over GSR patterns can both obscure imaging for photonic methods and impede the transfer of residues for chemographic methods.<sup>4-6</sup> Reagents that can liberate bloodstains without damaging GSR patterns have been described previously.<sup>4-6</sup> However, this study will address bloodstain removal specifically for photonic visualization. This is important for two reasons: incomplete removal of heme proteins is believed to liberate porphyrin compounds, which also fluoresce in the yellow-orange visible band.<sup>7</sup> Fluorescence from residual porphyrins could mask the desired fluorescence of GSR patterns. And, unlike previous studies using chemographic processing, the resolution and non-destructive nature of photonic GSR imaging lends itself as a quantitative research tool for evaluating results. The ideal blood removal reagent would be suitable for both photonic and chemographic methods.

Three blood removal reagents were tested and evaluated for their performance at varying parameters, including reagent volume, soak time, and blood deposit concentration. A Design of Experiments (DOE) approach was implemented to analyze the interactions between parameters and the results of those combinations. In total, 24 combinations of parameters were tested, with 8 trials for each blood-removal reagent and each trial performed in quadruplicate.

The GSR patterns were visualized using blue-orange fluorescence (450nm excitation/630 barrier filter) and infrared reflectance (850nm) prior to blood deposition. Following blood-removal processing, the GSR patterns were visualized again and processed chemographically as well. Photonic images were evaluated for propellant particle brightness-contrast, particle loss, blood removal, and loss of smoke/soot deposits. Particle loss was measured using Image Access<sup>®</sup> Forensic Analyzer software. Propellant particle brightness-contrast, blood removal, and loss of smoke/soot were measured using Adobe<sup>®</sup> Photoshop<sup>®</sup>. Chemographic results were evaluated for blood removal efficiency as well.

### Reference(s):

1. Meng, H.H.; Caddy, B. Gunshot residue analysis—A review. *J. Forensic Sci* 1997;42(4):553- 70.
2. Giroux, B. Non-destructive techniques for the visualization of gunshot residue. *AFTE J* 2006;38(4):327-38.
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### Gunshot Residue, Blood Removal, Blue-Orange Fluorescence

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## E116 Utilizing the ECHO Model to Virtually Train Medical Examiners and Coroners on Drug Overdose Investigations and Mortality Data Improvement

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**Learning Overview:** The Association of State and Territorial Health Officials (ASTHO) received funding from the Centers for Disease Control and Prevention (CDC) to pilot a virtual training for Medical Examiners and Coroners (ME/Cs) to improve reporting of drug overdose deaths. Project ECHO:OD-FIT (Overdose Fatality Investigation Techniques) is the first large-scale training opportunity created specifically to engage ME/Cs in discussion about their response to the overdose crisis. Training and requirements for ME/Cs vary greatly across the United States, leading to inconsistency in how overdose deaths are reported. OD-FIT uses the ECHO model to fill ME/C training gaps and allow a virtual space for best practices and experience to be shared. Many ME/C offices in the United States lack funding, resources, and time to provide ample training for staff, and this shortage of opportunities has been exacerbated by the increase in drug overdoses during the COVID-19 pandemic.

This presentation will explain the ECHO model and lessons learned from the pilot year of ECHO: OD-FIT. Attendees will learn the format of each session, the continuous quality improvement process, evaluation results, and lessons learned. They will also understand the importance of accurate death reporting and how this affects the public health response to the overdose crisis and emerging disease trends. This ground-breaking and innovative training series on drug overdose is the first of its kind and offers opportunities for expansion or replication to other audiences.

The success of this ECHO series for a difficult-to-reach population can be replicated for other forensic science populations. As the COVID-19 pandemic has continued, many trainings have been canceled or translated to virtual learning. The ECHO model was specifically created for a virtual platform and provides access to training, colleagues affected by the pandemic, and those who work in rural and secluded areas.

**Impact Statement:** ME/Cs are vital to United States public health surveillance, certifying and reporting more than 20% of yearly total deaths in the United States.<sup>1</sup> As the overdose epidemic has continued in the United States, it is imperative for public health decision makers to understand current and emerging drug overdose trends and where they are occurring. Having accurate and complete death certifications assists public health decisions by providing local, state, and national mortality data. This data can then be used locally to distribute resources to prevent overdose deaths. The forensic science community must understand this delicate relationship and continue to improve data.

A study in Vermont found over half of completed death certificates had errors or major errors and 93% had incorrect International Statistical Classification of Diseases and Related Health Problems, 10<sup>th</sup> Revision (ICD-10) codes.<sup>2</sup> This negatively impacts the quality of local and national mortality data and highlights the importance of continuous training. Another study found that 20.3% of death certificates indicting an overdose death in 2016 did not include a specific drug and had more missing information when filled out by coroners instead of medical examiners.<sup>3</sup> Project ECHO:OD-FIT is addressing that data gap by training ME/Cs to fill out death certifications completely and specifically. It is imperative for the forensic community to understand the importance of continuous training and improvement for practitioners to protect our nation's health. We must continue to engage providers with less resources virtually and effectively, even when in-person meetings resume.

This presentation will benefit any person or organization who wants to effectively train forensic practitioners virtually. Attendees may be considering new and innovative ways, like this ECHO model, to engage the forensic science community. This presentation will also highlight the important and often ignored significance of ME/Cs in public health surveillance.

### Reference(s):

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### Coroners, Training, Data



## E117 Are Medical Students Prepared to Recognize Child Abuse and Neglect? The Evaluation of an Italian Sample

Francesco Lupariello, MD\*, University of Turin, Turin, Italy; Francesca Capello, MD, University of Turin, Turin, Italy; Camilla Bonci, MD, University of Turin, Turin, Italy; Giancarlo Di Vella, MD, PhD, University of Turin, Turin, Italy

**Learning Overview:** After attending this presentation, attendees will have learned about Italian medical students' knowledge and training on child abuse and neglect.

**Impact Statement:** This presentation will impact the forensic science community by analyzing the degree of medical students' preparation on child abuse and by evaluating the medical school contributions on this topic.

Child abuse and neglect are widespread social and medical issues that often go unreported, despite being associated with significant morbidity and mortality.<sup>1</sup> They represent heterogeneous phenomena that widely vary in type, severity, and chronicity and, as a result, are challenging to identify: misdiagnosis may result in the development of psychiatric and other medical disorders.<sup>2,3</sup> In the scientific literature, a limited number of studies have been conducted about health workers' abilities to recognize suspected child abuse, although some researchers also reported physicians' lack of knowledge on this topic.<sup>4</sup> It is not clear if the reasons for this problem rely on insufficient preparation of students during medical school and/or a deficiency in continuing medical education during/after fellowships. For these reasons, a questionnaire was administered last year to medical students in order to evaluate the degree of knowledge on this theme. The study population included 179 Italian students, divided into two groups: Group A (79.8%)—students who attended pediatric traineeship and/or pediatric exam, and Group B (20.2%)—students who did not attend pediatric traineeship and/or pediatric exam. There were 12 questions focused on child sexual abuse, physical maltreatment, and neglect. Students' knowledge was considered as sufficient if the final score was  $\geq 6/12$ . Quantitative and statistical analysis were carried out by Excel<sup>®</sup> formulas.

Results pointed out an overall negative outcome for the most part of the 179 students (77.7% scored  $< 6/12$ ). In this population, medical students' awareness of child anogenital anatomy/pathophysiology appeared particularly deficient; 55.9% incorrectly answered that rectal prolapse is a manifest sign of chronic anal penetration. Indeed, a marginal percentage (7.3%) of students was aware of the physiological aspect of adult and preadolescent hymens. Similar considerations were highlighted considering the knowledge of suspected trauma sentinel lesions (abusive or accidental).<sup>5,6</sup> For all questions, results of chi-square tests yielded no statistical differences between the two groups: the deficiencies were not related to students' different levels of progression in medical school training and/or preparation. Therefore, this research clearly objectivated the need of a systematic implementation of basics in pediatrics, allowing specialists to acquire notions on child abuse and neglect.

The most significant limitation of this study relies on the difficulty of extending the abovementioned considerations to medical students from other geographical regions and/or countries. However, comparison of different medical school systems suggests common issues in students' education on child abuse and neglect.<sup>7</sup> Thus, all these systems are at risk of generating students and future medical doctors who are unable to attend to the responsibility of protecting children from abuse and neglect.<sup>8</sup> Study results and literature data suggested a widespread need to implement child abuse and neglect knowledge in medical schools. The present study highlighted the necessity for specific corrective interventions by governments and/or supranational organizations, as well as a proper pediatrics training.

### Reference(s):

1. Schilling, S.; Christian, C.W. Child physical abuse and neglect. *Child Adolesc Psychiatr Clin N Am*. 2014;23(2):309-319, ix.
2. Nemeroff, C.B. Paradise lost: The neurobiological and clinical consequences of child abuse and neglect. *Neuron*. 2016;89(5):892-909.
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8. Christian, C.W. Professional education in child abuse and neglect. *Pediatrics*. 2008;122 Suppl 1: S13-17.

### Child Abuse, Neglect, Medical Students

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## E118 Characterizing the Use of Blind Verification and Blind Proficiency Testing at Forensic Laboratories

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**Learning Overview:** After attending this presentation, attendees will understand the frequency with which laboratories are implementing blind verification and proficiency tests

**Impact Statement:** As interest in incorporating blinded procedures has increased, in conversations with laboratory management and Quality Assurance (QA) staff, there have been inquiries about practices at other laboratories. This presentation will help assess the state of the practice in the community.

The 2014 Bureau of Justice survey of publicly funded forensic crime laboratories found that while 97% of the country's 409 public forensic labs reported using some type of proficiency testing, only 10% reported using blind tests.<sup>1</sup> Further, local or state laboratories were far less likely to be conducting blind tests than federal laboratories. In October 2018, the Center for Statistics and Applications in Forensic Evidence (CSAFE), with the Allegheny County Office of the Medical Examiner, hosted a meeting with representatives from seven forensic laboratory systems, ranging in size from a single laboratory with fewer than 50 employees to a seven-laboratory system with over 200 employees, to assess interest in implementing blind proficiency testing and found significant interest as well as numerous obstacles.<sup>2</sup> In recent years, an increasing interest in implementing blinding in analyst proficiency tests as well as components of case work, such as verification of findings, has been observed at laboratories of different sizes and in different jurisdictions. To help characterize the degree to which these methods are being implemented, websites of forensic laboratories that are accredited by the ANSI National Accreditation Board (ANAB) for latent print analysis are being reviewed to identify those that publish their Standard Operating Procedures (SOPs) publicly. Specifically, SOPs related to proficiency testing and latent print processing, comparison, and verification are being reviewed. To date, 23 SOPs have been collected. Being recorded are:

- if latent print analysis is performed;
- if blind proficiency tests are used;
- if so, the frequency with which analysts complete blind proficiency tests;
- if verification is performed for conclusions of a match or non-exclusion;
- if verification is performed on other cases, and, if so, with what frequency;
- if blind verification is performed for conclusions of a match or non-exclusion;
- if blind verification is performed on other cases, and, if so, with what frequency;
- whether and how verification is reported;
- the type of jurisdiction for the lab (local, regional, state, national, other).

After the data has been characterized, follow up with select laboratories will occur to request interviews to discuss the lab's policies, when they were implemented, and, if there have been changes in recent years, the reasons for the change. In initial background interviews conducted as part of designing the study, directors and QA staff at forensic laboratories expressed interest in understanding policies on verification and proficiency testing at other laboratories; therefore, strong interest in the findings from this study is expected.

### Reference(s):

- <sup>1</sup> Burch, A.M.; Durose, M.R. Publicly Funded Forensic Crime Laboratories: Quality Assurance Practices, 2014. *Bureau of Justice Statistics* 2016:11.
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### Blinding, Verification, Proficiency test

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## E119 A Million Little Pieces: Pursuing Justice Through Methodology in Evidence

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**Learning Overview:** The goal of this presentation is to show how the multidisciplinary interactions led by a scientific forensic method play a substantial role not only to identify the cause of death but, above all, to serve justice.

**Impact Statement:** This presentation will have an effect on forensic pathologists by underlying the importance of a complete multidisciplinary approach in forensic practice. The cases proposed, in fact, demonstrate how the forensic pathologist can no longer be satisfied with the evidence deriving only from the macroscopic autopsy data, but with the integration of data deriving from a rigorous scientific method, if the truth is to be made explicit. (Pursuing Justice Through Method in Evidence.)

The heart of research in the field of forensic epistemology is research on the scientific method in the analysis and collection of case-specific evidence. This complex process cannot be separated from the exploration and experimentation of forensic sciences, especially in consideration of the goal of forensic activity, namely the search for truth. Therefore, it is no longer possible to be satisfied with only macroscopic data; in this regard, some cases are presented in which the execution of further investigations made it possible to completely change the supposed cause of death that emerged from the autopsy data.

The first case concerns the autopsy of a man involved in a road accident, found outside his vehicle. The autopsy macroscopic examination revealed the presence of an aortic transection at the isthmus level typical of the acceleration-deceleration dynamics, which affects the occupants of a vehicle involved in a high kinetic-energy collision. However, the study of the crime scene and the examination of the vehicle involved in the accident made it possible to collect evidence that led to the suspicion of being run over by a pedestrian. This last orientation of the investigations made it possible to determine that it was a murder committed by the son of the deceased for inheritance reasons.

The second case concerns a known criminal who fell into the water during a boat trip and was found floating near the boat. The circumstantial data was due to drowning, further supported by the absence of external damage detected by the forensic pathologist who intervened on the spot. The toxicological examination confirmed the presence of high concentrations of alcohol and cocaine in the blood. However, the Computed Tomography (CT) examination performed before the autopsy made it possible to identify the presence of the fracture of the tooth and axis according to a compression-extension mechanism, completely modifying the hypothesis on the cause of death.

The third case concerns an officer of an armed body who was found in the car compartment of his home, on the ground. The medical history was of the recent onset of acute low back pain for which he was being treated with intramuscular Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). At the autopsy table, the hypothesis was of sudden cardiac death. However, the subsequent toxicological investigation made it possible to identify traces of cocaine in the blood and urine, causally relevant to the cause of death, taken by muscle-popping.

The fourth case concerns a Tunisian man found suspended by means of a rope wrapped around his neck. The external examination was confusing: on one hand, the body was covered by purple bruises probably caused by external blunt force trauma; on the other hand, some typical signs of a suicidal hanging (such as the double ligature mark around the neck, swollen face, and protruding tongue) were also presented. Subsequent investigations identified a group of people who allegedly beat the subject, who were then investigated for murder. However, the lawyer claimed that they had caused the injuries in a fight a few days earlier and that the man then would have hanged himself in shame. However, immunohistochemical staining showed a dermal strong positivity to CD15, trypsinase, and IL-15 in the marginal zones above and below the hanging marks. Instead, the staining according to Perls' Prussian blue stain documented that the lesions produced dated back to less than 48–72 hours (the interval necessary for hemosiderin to form). They were then tried for murder.

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### Multidisciplinary Approach, Forensic Sciences, Pathology

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## **E120 Taking a Sniff Ahead: Updates on the Development of National Standards for Canine Detection Applications**

*Paola Prada-Tiedemann, PhD\**, Texas Tech University, Lubbock, TX; *Kenneth Furton, PhD*, Florida International University, Miami, FL; *Suzanne Perry, Old Dominion SAR, Sumner County, TN*; *Cynthia Otto, PhD*, University of Pennsylvania, Philadelphia, PA

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**Learning Overview:** After attending this presentation, attendees will understand the progression and status of national standards development for detector dogs and sensors and the plans for the completion, dissemination, and implementation of these standards, and the expected impact on the forensic science community, including improved location of and interdiction of forensic evidence and greater courtroom acceptance of dog alert evidence.

**Impact Statement:** This presentation will impact the forensic science community by providing a better understanding of how the Organization of Scientific Area Committees (OSAC) and the Academy Standards Board (ASB) of the American Academy of Forensic Sciences (AAFS) is improving the consistency in the training, performance, and certification procedures of operational detector dog teams and their integration as a forensic detection tool.

This presentation includes the results of the latest work in drafting detection-specific standard documents and working with a Standard Development Organization (SDO) to publish dogs' and sensors' standards to be used by various agencies, including law enforcement, the military, and homeland security. There are a variety of factors that influence the measured performance of canine teams, including the source of training materials, the containment system used, how the training materials are presented to detection teams, and how the teams were trained and how they are maintained. This initiative was undertaken as a response to concerns coming from a variety of sectors regarding the need to improve the performance, reliability, and courtroom defensibility of detector dog teams and their optimized combination with electronic detection devices. This presentation will describe the development of canine training standards over the past 16 years, beginning with the establishment of the Scientific Working Group on Dogs and Orthogonal Detector Guidelines (SWGDOG) in 2004, which by 2014 had completed 24 guidelines totaling 436 pages, to the guidance materials currently being developed by the Dogs and Sensors subcommittee of OSAC, which began in 2014, through the standard development process with the ASB beginning in 2016 and options for encouraging and certifying implementation of published standards, including the formation of the International Commission on Detector Dogs (ICODD). These efforts have been federally funded over the years by the National Institute of Justice (NIJ), the Federal Bureau of Investigation (FBI), the Department of Homeland Security (DHS), the Technical Support Working Group (TSWG), and currently the National Institute of Standards and Technology (NIST).

Since its inception, the OSAC – Dogs and Sensors subcommittee has identified 28 OSAC standards and technical reports making their way through the process map for approval by the ASB Consensus Body and, ultimately, OSAC approval for placing on the federal registry. These 28 documents relate to other related forensic disciplines. One technical report and one standard have already been successfully published by the AAFS Standards Board: Dogs and Sensors Terms and Definitions and General Guidelines for Training, Certification, and Documentation of Canine Detection Disciplines. The following standards are awaiting the final publication process through the Dogs and Sensors ASB: Dogs and Sensors Standard for Training of Pre-scented Canines Aged Trail Search; Dogs and Sensors Standard for Training of Pre-scented Canines Location Check; Dogs and Sensors Standard for Training and Certification of Canine Detection of Humans: Patrol Canine Team; Dogs and Sensors Standard for Canine Selection, Kenneling, and Healthcare.

The success and efforts of the Dogs and Sensors subcommittee is dependent on the members and affiliates utilizing the input from the community to help inform and shape these important standards to be ultimately implemented across the wide range of detection threats and needs.

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### **Detector Dogs, OSAC, Standards**

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## **E121 Standards Development Activities in Medicolegal Death Investigation**

*Kelly A. Keyes, BS\*, RTI International, Research Triangle Park, NC*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of medicolegal death investigation.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities related to medicolegal death investigation. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in medicolegal death investigation will be discussed. These include:

Recent standards that have been added to the OSAC Registry (ANSI/ASB Best Practice Recommendation 094, Postmortem Impression Recovery: Guidance and Best Practices for Disaster Victim Identification, First Edition, 2021)

Other OSAC proposed standards (ANSI/ASB Best Practice Recommendation 006, Best Practice Recommendations for DNA Analysis for Human Identification in Mass Fatality Incidents, First Edition, 2019; ANSI/ASB Best Practice Recommendation 007, Postmortem Impression Submission Strategy for Comprehensive Searches of Essential Automated Fingerprint Identification System Databases, First Edition, 2018; ANSI/ASB Best Practice Recommendation 009, Best Practice Recommendations for the Examination of Human Remains by Forensic Pathologists in the Disaster Victim Identification Context, First Edition, 2019; ANSI/ASB Best Practice Recommendation 010, Forensic Anthropology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority, First Edition, 2018; 2021-N-0007; Media Communications Following a Mass Fatality Incident: Best Practice Recommendations for the Medicolegal Authority; Victim Accounting: Best Practice Recommendations for Medicolegal Authorities in Mass Fatality Management)

Published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process (BPR 008: Mass Fatality Scene Processing: Best Practice Recommendations for the Medicolegal Authority; BPR 094: Postmortem Fingerprint Recovery: Guidance and Best Practices for Disaster Victim Identification)

Documents currently in development at the OSAC or by the Academy Standards Board (ASB) (Workload limitations for Medicolegal Death Investigators; Best Practice Recommendation on MDI Scene Response; Best Practice Recommendations for Interactions with Next of Kin; Best Practice Recommendations for Interactions with Organ Procurement Organizations; Best Practice Recommendations for Fatality Management During a Pandemic; Standard for Disaster Victim Identification; Forensic Odontology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority; Organizational and Foundation Standard for Medicolegal Death Investigation)

Priorities for new documents or work products (Commonly exchanged data in medicolegal death investigation; Family Engagement Following a Mass fatality Incident; Victim Information Center Best Practice Recommendations for Medicolegal Authority; Mass Fatality Management During a Pandemic; Best Practice Recommendations for Managing Disaster Victim Identification with Chemical, Biological, Radiological, Nuclear, and Explosive Trauma; Ethical Considerations in Disaster Victim Identification) and other highlights, including reference documents [newly published Principles to Promote Research in Medicolegal Death Investigation and Medicolegal Death Investigation Data Commonly Collected and Exchanged] and a glossary).

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### **Medicolegal Death Investigation, Standards, OSAC**

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## E122 Evidence Management and the Forensic Lab: Results From a Nationwide Survey of Evidence Handlers

*Shannan Williams-Mitchem, MPP\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Overview:** This presentation will discuss results of the Evidence Management Survey with special emphasis on survey respondents who manage evidence at forensic laboratories. After attending this presentation, attendees will have learned about the current state of evidence management personnel, training, policies, and practice in forensic labs in comparison to other organizations involved in the management of physical evidence based on results from the Evidence Management Survey administered by the National Institute of Standards and Technology (NIST) and in close consultation with members of the Evidence Management Steering Committee (EMSC), a multidisciplinary group co-funded by NIST and the National Institute of Justice (NIJ).

This presentation will provide a summary of the findings in survey results with special emphasis on respondents from crime labs. An overview of EMSC activities, published reports, and upcoming activities will also be discussed.

**Impact Statement:** Maintaining the integrity of physical evidence is a fundamental aspect of the administration of justice. Every day, critical decisions are made to determine outcomes in legal proceedings based on evidence and how it is managed. Once identified as evidence, the item begins a long and often-complex journey until its final disposition (e.g., destruction, return to owner). Short- and long-term maintenance of the evidence involves a wide range of stakeholders, each with different organizational roles and responsibilities in the context of the larger system of justice. The forensic laboratory is just one of the critical organizations involved in the management of evidence.

In 2018, the EMSC was formed by NIST to develop best practices for the storage and preservation of physical evidence. One of the primary goals of the committee was to assess the state of the evidence management industry by performing a gap analysis of evidence management needs. The Evidence Management Survey was developed as one method to learn about evidence handlers, the organizations they work in, and the existing practices that enable them to retain, preserve, track, and dispose of evidence items.

Each inquiry in the 85-question instrument was carefully considered, vetted, and piloted in numerous discussions for over a year. Per federal regulations, the survey was approved for release by the Office of Management and Budget (OMB) and in accordance with NIST Institutional Review Board (IRB) requirements on January 12, 2021. The survey was widely distributed through multiple channels, including organizations represented by EMSC, NIST, NIJ, RTI International, and many others. The development of the electronic version of the survey and results analysis was supported by RTI International. The survey was closed on April 16, 2021.

There was a total of 1,443 responses to the Evidence Management Survey. Most respondents (51.42%) were from law enforcement agencies with the crime/forensic laboratory (26.96%) agency type being the second most common. Other agency types represented included medical examiner/coroner offices (7.42%), private industry (5.13%), court systems (4.92%), clinic/health care (3.74%), and correctional systems (0.42%)

This presentation will provide a summary of the findings in survey results with special emphasis on respondents from crime labs. An overview of EMSC activities, published reports, upcoming activities will also be discussed.

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### Evidence Management, Preservation, Storage

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**E123 A National Perspective of How Unidentified Human Remains Are Handled in the United States Using a 2018 Census of Medical Examiner and Coroner Office Data**

*Ashley Rodriguez\**, RTI International, Research Triangle Park, NC; *Kelly Keyes*, RTI International, Research Triangle Park, NC; *Christopher Williams*, RTI International, Research Triangle Park, NC; *Donia Slack*, RTI International, Research Triangle Park, NC; *Stirling Cummings*, RTI International, Research Triangle Park, NC; *Jeri Roper-Miller, PhD*, RTI International, Research Triangle Park, NC; *Patricia Melton*, RTI International, Research Triangle Park, NC

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**Learning Overview:** This presentation will discuss the findings of the 2018 Bureau of Justice Statistics' (BJS) Census of the Medical Examiner and Coroner Offices (CMECs), specifically focused on policies and procedures related to the handling of Unidentified Human Remains (UHRs) and the impact those policies and procedures have on the extent of UHR cases within the United States. At the end of this presentation, attendees will have a grasp of the most current, systematic information known about the extent of UHRs in the United States, and the resources, policies, and practices MECs use to investigate and track these cases.

**Impact Statement:** This presentation will impact the forensic community by highlighting the extent of UHR cases in the United States and demonstrating how policies and practices implemented by the MEC community have affected these cases.

In 2019, the United States BJS administered the CMEC, which included questions about how UHR cases were handled in 2018. In addition, agencies provided insights on policies regarding the retention of UHR evidence and reports as well as their use of national databases such as the National Missing and Unidentified Persons System (NamUs), National Crime Information Center (NCIC), and Combined DNA Index System (CODIS) in UHR investigations.<sup>1-3</sup> More than 1,600 agencies responded to the census request.

In 2019, RTI International conducted the 2018 CMEC on behalf of BJS (2017-MU-CX-K052). A questionnaire was designed, reviewed by a forensic expert panel, and piloted with select MEC offices. The census was fielded through mail-out, email correspondence and follow-up Computer-Assisted Telephone Interviewing (CATI). The MEC offices provided responses via mail, facsimile, CATI, and a web-based instrument. Questions focusing on the handling of unidentified human remains cases were analyzed and will be discussed. Where appropriate, selected comparisons were made with the 2004 CMEC data.

The results from this study provide insight into the policies and procedures enacted by MECs nationwide to handle UHR cases. Overall, the responding agencies had fewer UHR cases on file at the end of 2018 when compared to UHR cases reported during the 2004 CMEC administration. Key findings indicate most agencies do not have retention policies in place for evidence related to UHRs, and national database resources that have proven to aid in identifications have historically been underutilized by the MEC community.

The implications of the 2018 results will be discussed as they relate to the resolution of UHR cases, which point to needed resources, policy, and practice shifts. Lastly, limitations of the Census will be discussed and suggestions for future studies will be provided.

**Reference(s):**

1. National Missing and Unidentified Persons System (NamUs) is a national resource center for missing, unidentified, and unclaimed persons. <https://namus.nij.ojp.gov/>.
2. National Crime Information System (NCIC) is a central repository of missing persons and criminal information that allows for the rapid sharing of information between criminal justice agencies. <https://irp.fas.org/agency/doj/fbi/is/ncic.htm>.
3. Combined DNA Index System (CODIS) is the U.S. national DNA database maintained by the FBI in support of the criminal justice system. <https://www.fbi.gov/services/laboratory/biometric-analysis/codis#CODIS-Overview>.

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**Unidentified Human Remains, Medical Examiner and Coroner Offices, Evidence Retention Policies**

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## E124 Variation in Laboratory Policies and Procedures Related to Interpretation of DNA Mixtures

*R. Austin Hicklin, PhD\*, Noblis, Reston, VA; Nicole Richetelli, PhD, Noblis, Reston, VA; Mitch Holland, MS, Noblis, Reston, VA; Robert Bever, PhD, Bode, Lorton, VA; Lauren Brinkac, MS, Noblis, Reston, VA; Jonathan Davoren, MS, Bode, Lorton, VA*

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**Learning Overview:** After attending the presentation, attendees will have learned about the variability of policies and procedures between laboratories in analyzing DNA mixtures.

**Impact Statement:** This presentation will communicate the documented inter-laboratory variability in the policies and procedures to interpret DNA Mixtures.

The purpose of this presentation is to describe the first phase of the ongoing study, *Inter-laboratory Variation in Interpretation of DNA Mixtures* (NIJ Grant 2020-R2-CX-0049). The results of the Policies and Procedures (P&P) Questionnaire and variability in laboratories' standard operating procedures related to DNA mixtures will be presented. The study is being conducted to evaluate the current state of the practice of DNA mixture casework and is not restricted to specific products or statistical approaches.

Since 1995, the mixture interpretation process has been continually improved through numerous research and development efforts. Within the past five years the use of probabilistic genotyping has become common in laboratories as it significantly advances the mixture interpretation process. Almost all inter-laboratory studies reported to date were conducted prior to the widespread adoption of probabilistic genotyping software in crime laboratories. This study evaluates the current state of the practice in interpretation of DNA mixtures utilizing either binary or probabilistic genotyping protocols. The scope is limited to variability in interpretation and analysis of electropherograms; thereby eliminating variability due to laboratory processing of physical samples. The project expands on the results and lessons learned from DNA mixture inter-laboratory studies conducted to date, most notably the National Institute of Standards and Technology (NIST) MIX13 study, and address concerns regarding complex DNA mixtures raised by the President's Council of Advisors on Science and Technology (PCAST) Report on Forensic Science in Criminal Courts in 2016.<sup>1</sup>

The study is composed of four phases conducted to assess the sources of variability in analyzing DNA mixtures:

- Policies and Procedures (P&P) Questionnaire—Online questionnaire to assess laboratory policies and procedures relevant to DNA mixture interpretation (notably systems, types of statistics reported, and parameter settings used).
- Casework Scenario Questionnaire—Online questionnaire presenting a number of casework-derived scenarios (without DNA data), asking participants to assess how they would conduct analysis for each scenario.
- Number of Contributors (NoC) Subtest—Assessment of suitability and number of contributors, given electropherogram data for 12 mixtures.
- Interpretation, Comparison, and Statistical Analysis (ICSA) Subtest—Interpretations and statistical analyses, given electropherogram data for eight mixtures, each provided with DNA profiles of potential contributors.

### Reference(s):

- <sup>1</sup>. 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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### DNA Mixtures, Probabilistic Genotyping, Inter-Laboratory Variation



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## E125 Toward the Quantitative Evaluation of Pattern Evidence

*Alicia Carriquiry, PhD\*, CSAFE-Iowa State University, Ames, IA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the calculation of the likelihood ratio in pattern comparison disciplines, of the correct interpretation of findings in forensic evaluations, and of the barriers that must be overcome to apply these methods in practice.

**Impact Statement:** This presentation will discuss strategies to implement quantitative methods in case work and will mention some of the barriers that need to be eliminated. The impact resides on the potential to begin the transfer of knowledge from theory to practice.

In the past several years, forensic scientists offering testimony in court about pattern comparisons have faced challenges from lawyers invoking provisions in *Daubert*, the *Federal Rules of Evidence 702*, and similar provisions. While no judge has excluded pattern comparison testimony until now, the strength of the conclusions that examiners can report has been significantly curtailed in several recent cases involving firearm evidence.<sup>1,2</sup>

Examiners in the pattern comparison disciplines have been reluctant to adopt quantitative methods to analyze and interpret evidence. This is understandable; applying quantitative methods to data that consist of images is challenging and most of the algorithms that have been developed are not yet “mature” enough or have not been tested sufficiently. Further, there has been spotty communication between the communities of forensic practitioners and of academic researchers, with the result that the exchange of knowledge has not been fluid, and adoption of quantitative methods has been slow.

This presentation addresses some of those challenges and offers suggestions for moving toward implementing quantitative methods in case work. This presentation first introduces the likelihood ratio approach using everyday language and intuitive ideas. Using various comparison disciplines as illustration, the discussion will then focus on *implementation* issues: What measurements can be obtained from pattern evidence? How can those measurements be used to calculate a likelihood ratio? How are the results interpreted correctly? To address those and other related questions, presenters will go over calculations using actual data obtained from bullet comparison studies and—time permitting—from footwear examination studies. In addition, the presentation will also discuss what is known thus far about the accuracy of the bullet comparison algorithm developed by Center for Statistics and Applications in Forensic Evidence (CSAFE) researchers as well as some of its limitations.<sup>3</sup> Even though the algorithm appears to be accurate (albeit after insufficient testing), there are barriers that must be sorted before it can be applied in practice. These barriers, and how to overcome them, will be discussed.

The transition from subjective evidence evaluations and categorical conclusions to data-reliant, probabilistic conclusions is likely inevitable, but the new quantitative era will not be ushered in until communication and meaningful collaboration between practitioners and researchers become more fluid and fruitful. Recent black-box studies conducted in several disciplines have resulted in low estimated error rates, which may suggest to some that perhaps the current subjective approaches to evaluate pattern evidence will no longer be questioned. Rather, a worthy goal may be to combine the best of both approaches to optimize the performance of examiners. This presentation will propose strategies to pursue that goal.

### Reference(s):

- <sup>1.</sup> *State v. Pickett*, 246 A.3d 279, 466 N.J. Super. 270 (Super. Ct. App. Div. 2021). 2021.
- <sup>2.</sup> *United States v. Tibbs*, 2016 CF1 19431, (Super. Ct. District of Columbia, 2016). 2016.
- <sup>3.</sup> Hare, E.; Hofmann, H.; and Carriquiry, A. Automatic matching of bullet land impressions. *The Annals of Applied Statistics*. 2017; 11(4): 2332-2356.

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### Quantitative Methods, Pattern Comparison Disciplines, Implementation and Barriers

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## E126 Border Police Officers Versus Automatic Border Control Systems

*Andrijana Filko\*, Croatian Forensic Science Centre i.Vucetic, Zagreb, Croatia*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the problematic of Automated Border Control Systems. Through the several possible scenario at the border crossing points that has been shown in this research, attendees will be learning how human factors impact on border control in general as well as how the use of Automated Border Control Systems may become a risk, reducing instead of increasing border security.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness that Automated Border Control Systems alone are not sufficient to identify the impostors or look-alikes, especially regarding the facts that the most dangerous ID fraud involves impostors capable of fooling even the most advanced of today's facial recognition systems.

Look-alike fraud is one of the most common phenomena of fraud by abusing someone else's identity, whereby the holder of the document physically resembles the owner of the document. The main goal of this research was to indicate the advantages and disadvantages of Automated Border Control Systems in the light of misuse and/or forgery of (ID and) travel documents, as well as to point out new modalities of forgery (*modus operandi*). Nowadays, (ID and) travel document fraud is an important and growing problem connected with different forms of cross-border criminal behavior such as illegal immigration (including human trafficking), drug trafficking, money laundering, firearms smuggling, and terrorism. Border police officers almost daily encounter persons possessing two or more original travel documents of different issuing states and different names of the document holders. According to unofficial estimates, thousands of people with double or multiple identities cross the international borders daily by using authentic documents based on fraudulent identity.

The importance of this research is the awareness of an indisputable fact that the nature of (ID and) travel document fraud is evolving rapidly, involving new *modus operandi* and new forms of forgery to circumvent biometric checks. Based on a number of examples from this research as well from FRONTEX (the European Border and Coast Guard Agency) research, it is possible to conclude that the best way to prevent (ID and) travel document fraud is a technical and tactical approach (document check versus profiling) developed in tight cooperation with forensic document experts, who can provide any additional training on demand.

The main result of this research has shown that based on profiling and knowledge of *modus operandi*, it is possible to detect high-quality forged documents that are impossible to be detected by an automated verification device. Consequently, the main conclusion of this research is that it is necessary to continuously invest in the professional knowledge, skills, and competencies of border police officers, who will then, with the use of appropriate equipment and aids, successfully detect forged documents and impostors.

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### Document Fraud, Impostors, Automated Border Control Systems

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## **E127 New Jersey’s Drug Monitoring Initiative: Developing Forensic Intelligence to Identify Drug Trends, Support Law Enforcement Investigations, and Share Information With Laboratory and Public Health Partners**

*Adam Polhemus, BA\*, New Jersey State Police Drug Monitoring Initiative, West Trenton, NJ; Jason Piotrowski, BS, New Jersey State Police Drug Monitoring Initiative, West Trenton, NJ*

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**Learning Overview:** After attending this presentation, attendees will be able to: (1) outline the need for building a Drug Monitoring Initiative (DMI) capability, based on forensic laboratory data and other key public safety and public health data sets, to identify drug trends and inform all relevant partners; (2) identify how the DMI utilizes forensic intelligence to support law enforcement investigations, interrupt the drug supply, and reduce community drug harms; and (3) recognize how monitoring and sharing forensic laboratory data allows forensic scientists and toxicologists awareness of new illegal substances and unique drug forms, contents, packaging, and emerging drug trends.

**Impact Statement:** New Jersey’s DMI, the leading drug environment monitoring concept, enhances public safety and public health through an in-depth knowledge base that provides real-time awareness of drugs impacting New Jersey. Working intimately with statewide forensic laboratory data and integrating other key public safety and health data sets, the DMI compiles a robust collection of drug-related information. Leveraging current technologies, processes, and analytical capabilities allows for analysis and the DMI to develop forensic intelligence. This intelligence leads to: identification of emerging drugs and poly-drug adulteration; supporting law enforcement investigations and identifying connected drug cases; producing significant analytical products; informing nationwide forensic laboratory partners; and providing awareness to multiple community drug harm and multi-sector outreach initiatives.

DMI collects law enforcement narcotic seizures and overdose drug evidence information submitted to and analyzed by New Jersey laboratories. DMI analysts track spikes in overdoses, create a temporal analysis of where spikes are occurring while identifying the dangerous substances involved. In addition to the significant historical laboratory data DMI holds, New Jersey forensic laboratories will expedite analysis to confirm the substance’s contents. DMI distributes intelligence products that identify these deadly brands and locations to transition narcotic operations to locate and dismantle the illicit activity and consumption of deadly substances.

Additional production of real-time drug-related alerts, warnings, and intelligence products are derived by continuously monitoring laboratory data and sharing forensic intelligence to multi-sector partners pertaining to the state’s drug environment, thus enabling not only situational awareness of drugs analyzed, but pro-active deployment of investigative, prevention, and treatment resources to at-risk areas, populations, and individuals.

Recognized by the National Governor’s Association, Office of National Drug Control Policy, and National High Intensity Drug Trafficking Area (HIDTA) as a nationwide best practice, the DMI initiative establishes an intelligence capacity allowing law enforcement and public health experts to better understand trends, implications, and threats from illicit drug activity having an impact within their jurisdictions. The DMI partnership represents good government, as disparate agencies are sharing information and collaborating to fight the illicit drug epidemic that New Jersey and other states in the region are facing. The combination of law enforcement data and forensic laboratories analysis with health care data has proven to be an effective tool to better understand the state’s drug environment at micro and macro levels. The core data elements that provide a timely picture of the drug environment are those derived from drug seizures and drug overdoses. Access to these data sets in real-time enables understanding of the drug environment for emergent and strategic purposes. These data sets provide the DMI partners with the ability to be informed regarding overdose incidents, enabling them to better prepare for, respond to, and prevent overdose incidents in their jurisdictions. These data sets allow the DMI to provide the scientific community drug environment information related to new illegal substances; drug packaging, forms and trends; and presumptive and confirmatory analysis encountered in the field or laboratory. Long term, these data sets have enabled DMI partners to better understand the prevalence and patterns of substance abuse to develop effective substance abuse prevention strategies.

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### **Intelligence, Investigations, Sharing**

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**E128 How Educators in the American Academy of Forensic Sciences (AAFS) Have Met and Surpassed the Challenges of Providing Forensic Science Education During COVID-19 to Produce Improved Education and Training for the Modern Forensic Science World**

*Stewart Walker, PhD\*, Flinders University, Adelaide, South Australia, Australia*

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**Learning Overview:** Attendees will consider examples of better practice in forensic science education and training arising from changes required during COVID-19 conditions and compare them with their own experiences.

**Impact Statement:** The forensic science (education and training) community will benefit from discussions of potential ways in which they can improve the delivery, assessment, and impact of forensic science methods during a pandemic. This will encourage discussion and dissemination of other examples of alterations that will benefit the community.

During the COVID-19 pandemic, universities, and other teaching and training establishments have had to quickly respond to the challenges of delivering and assessing forensic science education. This presentation will outline some of the processes that have been implemented and how these have led to improvements in forensic science education that meet and surpass the challenges of teaching forensic science in the modern world. It is anticipated that when things go back to “normal,” forensic science education will incorporate some of the modern approaches with the best of the traditional approaches to produce a product that is better than it was pre-COVID-19.

During forced lock-downs and isolation, forensic science, like other education areas, has had to quickly adapt to delivery by remote means rather than traditional face-to-face methods. This requires consideration of new ways of material delivery, hands-on practical experiences, and assessment.

**Material Delivery:** It is relatively easy to deliver old-fashioned 50-minute lectures with PowerPoint® slides via a number of different programs for uploading and disseminating content. However, to just record a talking-head with slides is underutilizing the potential for these programs and is not using the new technology to its best advantage. Splitting the material up into smaller “bites” was necessary to prevent files from being too large, but this had the advantage in that material could be more aligned with attention spans. Also, the ability to incorporate movies and illustrations helped to break up the material. Platforms that enabled communication between lecturer and students also could be used to change the presentation from being a monologue to being a dialogue—or even a polylogue. Setting tasks for students to find some information or cases and report back had the double advantage of expanding the material from what the lecturer taught to bring in other material and also got the students engaged, active (rather than passive,) and gave them some ownership of the material.

**Hands-On Practical Experiences:** Forensic science, in common with other disciplines, requires hands-on activities to reinforce concepts and effectively train future practitioners. Recording a technician doing the practical and then supplying data for processing may be the only option available but is not sufficient to “train” the students in a hands-on way. Due to restrictions in numbers and spacing, practical’s have been redesigned to still provide a learning experience without the need for four students to sit around one piece of apparatus for three hours. Concentrating on the preparation and analysis of evidence by individual students rather than working in pairs to fours has improved the learning experience for all students.

**Assessment:** The necessity to have assessment done remotely and delivered through computers, rather than in exam rooms, has required the re-assessment of how assessment is carried out and what is being assessed. Exam questions now need to be “search-engine-proof” to prevent the answers being searched and regurgitated from the Web. This has meant that assessment can require the student to think more and assess and structure answers to real problems, which may be a better and more realistic way of assessing the student’s ability to work on real problems in a realistic way in the workforce.

It is anticipated that when (or if) conditions return to “normal,” the advantages of some of these new approaches to delivery and assessment will be retained and mixed with the best of old practices to produce forensic science education that is actually better than it was before the pandemic. In this way the educators in the Academy will have met and surpassed the challenges of providing forensic science education in the modern forensic science world.

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**Education, COVID-19, Improved**

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## E129 Integrating Cold Case Investigations in Forensic Science Education: Student Engagement in Research, Information Literacy, and Data Visualization

*Krystal Hans, PhD\*, Purdue University, West Lafayette, IN; Rachael Rosselet, MPhil, Walden University, Minneapolis, MN*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of cold case investigations and the incorporation of forensic science educational strategies in these investigations.

**Impact Statement:** The information in this presentation will impact the forensic science community by presenting a forensic science educational tool and a developing database of cold case information that incorporates the work of students, forensic scientists, law enforcement, and citizen detectives.

In a collaboration between Uncovered and Purdue University, students in forensic science courses are completing projects on cold case investigations to participate in research, critical thinking assignments, and information evaluation.<sup>1</sup> The concepts of research, information literacy, and data visualization are highlighted in the student projects, and students learn about the ethical concepts of digital detective work, the value of collective impact, and the importance of inclusive victimology. Students are assigned a cold case, watch videos and hear lectures from Uncovered, and participate in group research to develop a comprehensive overview of the people, timeline, locations, sources, tips, and theories related to the case and the investigation. Students participate in group and class discussions, complete assignments, and reflection surveys throughout the semester. The culminating deliverable is the production of a detailed case spreadsheet, as well as an infographic to display all pertinent information about the cold case and investigation. Data on student learning, thematic analysis of information sources, and student experience will be collected from reflection surveys and interviews.

Uncovered, a Public Benefit Corporation, is a new and innovative software platform that combines data, analytics, and the “wisdom of the community” to help solve the cold cases of murdered or missing people. Uncovered is developing a comprehensive nationwide database of unsolved missing and murdered cases. All information is assembled into a Digital Case File that contains a visualized timeline of events overlaid onto a map of locations, a catalog of the people involved, and an extensive reference list. Finally, each case contains clear and direct calls to action on how the public can help the victim.

Using the Graph Theory and the open-source Neo4j platform, Uncovered is building an analytics and data model that focuses intently on Persons, Objects, Locations, and Events.<sup>2</sup> This will allow for unstructured discovery of its data, letting users make connections that were not visualized previously. Uncovered has also curated an engaged community of citizen solvers and digital volunteers to crowdsource the gaps in unsolved cases. Members are provided research tools and resources, training, and access to experts and others with a shared passion to solve a mystery.

The goal of this research is to develop a sustainable framework of student and community involvement that can be transferred to other universities with forensic science and criminal justice programs. This research and collaboration provide opportunities for new avenues in forensic science education, preparing students professionally with the incorporation of real-world experience and engagement with the greater law enforcement and forensic science community. Additionally, this also provides a real-world application for students to see the impact of their work directly.

### Reference(s):

1. Uncovered. (n.d.). Cold case files for murdered or missing people. <https://uncovered.com>.
2. Donga, J.; Bhojak, P.; Patel, K.; Shah, V. An analysis of different computer science algorithms with the graph theory of mathematics. *Reliability: Theory & Applications*. 2021, 16(1): 376-384.

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### Experiential Learning, Cold Case, Forensic Science Education

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**E130 Examples and Lessons Learned From Virtual Reality Simulation Inclusion in Forensic Humanitarian Training**

*Ivett Kovari, PhD\*, ICRC, Geneva, Switzerland; Sherry Fox, PhD, ICRC, Geneva, Switzerland; Pierre Guyomarc'h, PhD, ICRC, Geneva, Switzerland; Melissa Kiehl, ICRC, Geneva, Switzerland; Christian Rouffaer, ICRC, Geneva, Switzerland; Xuan Wei, ICRC, Geneva, Switzerland; Kristy Winter, ICRC, Geneva, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the inclusion of virtual reality simulation in the training of first responders, national mechanism authorities, international organizations, and forensic practitioners.

**Impact Statement:** This presentation will impact the forensic science community by: (1) highlighting the complexity of management of the dead training for first responders, and (2) providing examples of experience-based contemporary training involving virtual reality simulations.

The emergence of technology has provided an increase in capability and functionality of forensics in the humanitarian sector. A series of digital tools, such as a COVID-19 chatbot and online courses, that have been developed by the International Committee of the Red Cross (ICRC) has addressed the emergency needs for improved response by forensic practitioners and for the benefit of the affected communities. One of the most beneficial emerging tools developed by the ICRC is the “management of the dead virtual reality simulation,” a scenario developed from the ICRC, World Health Organization (WHO), and Pan American Health Organization (PAHO) standard manual for first responders and based on forensic trainers’ experiences on in-person training courses.

The Management of the Dead in Disasters course is organized in response to the needs identified by the local authorities with the ICRC as a mutual effort. It introduces participants to standard and practical methods of managing the dead after an emergency, disaster, or catastrophic situation. The training course uses a multidisciplinary approach; however, a challenge faced in the training course as the role play simulation within a classroom environment limits the participant’s ability to engage with the task and the situation at hand. Virtual reality has revolutionized teaching when integrated into training, as it provides an immersive environment and multiple safe opportunities to practice the technical skills expected to be learned from the course in the course. Multiple training courses integrating the scenario into their course across multiple contexts (South Africa-, Thailand-natural disaster, and Armenia-natural disaster and post-conflict situations) and a wide range of participants (first responders, policing organizations, and Red Cross societies) have confirmed the added value of virtual reality in forensic training.

This presentation highlights the benefit of the inclusion of virtual reality simulations in forensic training and the challenges of using state-of-the-art technology for simulated environments.

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**Management of the Dead, Humanitarian Forensic Action, Virtual Reality**

## **E131 Mental Health and Resilience Education for Forensic Science Students**

*Karen Woodall, PhD\*, University of Toronto, Toronto, Ontario, Canada; Grace Gregory-Alcock, BSc, University of Toronto, Toronto, Ontario, Canada*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of introducing the topics of stress, resiliency, and Post-Traumatic Stress Disorder (PTSD) into the forensic science curriculum at the undergraduate level to help prepare the next generation of forensic science professionals.

**Impact Statement:** This presentation will provide the forensic science community with information on how to implement the topic of mental health issues into forensic science courses or training programs.

Recent research has shown the incidence of PTSD and mental health issues in the forensic community is significant and is likely to increase if it remains unaddressed.<sup>1,2</sup> People working in forensics often deal with unique stressors; they are exposed to traumatic material from forensic cases and often work within an adversarial legal system. Current research is beginning to address this issue and, consequently, the importance and urgent need for resiliency training for individuals working in forensic science and related fields is becoming recognized. In addition to forensic science professionals, it would be beneficial for students contemplating a career in forensics to be aware of the various types of traumas, stresses, and the potential impacts that they may have during their career. Increasing the awareness of the various types of traumas that forensic professionals routinely encounter, and reducing the stigma associated with discussing mental health is an important step in preparing the future employees in forensic and law enforcement community.

The University of Toronto, Mississauga, offers an undergraduate degree program in Forensic Science with the opportunity to specialize in Anthropology, Biology, Chemistry, and Psychology with many graduates pursuing careers in law enforcement and forensic laboratories. A recent addition to the core curriculum was implemented to provide education about mental health, resiliency, and useful strategies to mitigate and reduce the impacts of stress. Students learned about vicarious trauma, PTSD, and how they relate to forensic professions, and the effects that exposure to trauma and stress can have on personal and professional performance. In addition to learning about mental health and resiliency, students had the opportunity to hear experiences directly from professionals who have first-hand experience working within the forensic community.

Educating the next generation of forensic scientists, beginning at the undergraduate level, will help meet the health and wellness needs of employees, avoid burnout, reduce decreased work performance, and help create a positive work environment.

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### **Education, Resiliency, Curriculum**

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## E132 Understanding Foundational Ethical Content Necessary for Educating Future Forensic Scientists

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**Learning Overview:** After attending this presentation, attendees will understand the foundational ethical content described by active practitioners and educators as being necessary for educating future forensic scientists.

**Impact Statement:** This presentation will influence the forensic science community by highlighting the importance of ethics training and ethics education based on experiences from current practitioners and educators.

Generally, many people consider themselves to have a proficient understanding of ethics to work in their specific disciplines. Though this may be true sometimes, employers have reported a deficiency of ethics education in the workplace. Along with this deficiency, it has been noted that many people confuse ethics with morals. Given the requirement for ethics education by the Forensic Science Education Programs Accreditation Commission (FEPAC), the importance of ethics in forensic science can be observed. A lack of well-structured ethics courses steered toward forensic science calls for research to be conducted to investigate what should be included in such a course. The work completed focused on gathering information pertaining to ethical content that should be included in a foundational undergraduate ethics course directed toward forensic science majors. This work was completed in tandem with research being conducted to create course content for ethics education in forensic science.

This two-part study collected data from active forensic science practitioners and ethics educators. During Part One, interviews were conducted with a variety of ethics educators from a number of disciplines, including forensic science, theology, philosophy, business, and bioethics. Ethics educators who participated in an interview gave directions pertaining to which philosophers and philosophical ideas should be included in course content for ethics education in forensic science.

Results from interviews conducted with ethics educators indicated that philosophical ideas by Immanuel Kant, Aristotle, and David Hume should be included in course content aimed toward forensic science students. Additionally, it was determined that ethics is best taught through case studies and student engagement with course material.

Part Two included surveys and interviews completed by forensic scientists who were asked questions pertaining to experiences in the workplace and their understanding of ethics. Recruitment was conducted by an email sent to the American Academy of Forensic Sciences members of the Criminalistics section. Participants were invited to complete a survey through Qualtrics or an interview via Zoom. Questions asked pertained to the practitioner's experience related to ethics training, encountering unethical behavior in the workplace, and personal thoughts on the type of content necessary to educate future forensic practitioners. Those who participated in interviews were asked an additional question based on response from the survey.

Results from the survey indicate that 35% of participating practitioners have never encountered an ethical dilemma in the workplace. Moreover, it was observed that some practitioners do not believe that an improvement in ethics education would affect the ethical practices of future practitioners. Though many ethical dilemmas were mentioned through the survey, an overwhelming amount included attempted collusion by lawyers and prosecutors. Other dilemmas encountered included malpractice by laboratory management and co-workers. Over 85% of survey participants believe improved ethics education is important for future practitioners. Future direction of this research includes using the data to create ethics content for upcoming forensic scientists.

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### Ethics, Education, Behavior



**E133 Bias—It’s Not Just in Your Numbers: The Importance of Teaching Unconscious Bias in Forensic Education**

*Charla Skinner Perdue, MS, MFS\*, Florida State University, Panama City, FL*

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**Learning Overview:** The goal of this presentation is to provide forensic science educators and practitioners who train with the ability to identify their own implicit biases and develop tools to educate others to recognize everyone has unconscious biases. This presentation will provide attendees with strategies to keep bias from impacting decision making. Attendees will be encouraged to acknowledge steps in the forensic process that can be problematic and be inspired to rethink practices that may be negatively affected by human performance.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the role of the one analyzing or interpreting evidence and the benefits of developing well thought-out evidentiary procedures that mitigate the potential for implicit bias in the form of analysis subjectivity that impacts decision making in forensic science. The goal is for those in attendance to understand how the legitimacy of the criminal justice system requires unbiased judgement and their role in achieving it.

Neuroscientists have determined the brain uses bias to help humans quickly categorize information. Our brains will use old information we have learned, experiences we have had, past relationships, and all the other data we have on a subject and relate the past to new material received. It is a necessary function, and these created biases are not all bad. The problem is when these biases impact our decision making.

In forensic science, great strides have been made to mitigate bias from the scientific method. Despite the recent attention given to implicit bias and its unintentionality, most people still do not see themselves as biased. Many times, in forensic science, failure is caused by human performance, not process. The bias expressed in the subjectivity, discretion, and judgement of the expert in the form of interpretation partiality is where unconscious bias can impact decision making, and the area forensic science needs to continue to improve. The ability to accept bias in the procedure and not the decision maker is where good forensic education and training should begin.

Concentrating on the role of the one interpreting evidence and the benefits of developing well thought-out evidentiary procedures that mitigate the potential for implicit bias by the one performing the interpretation or analysis of the evidence will enhance outcomes and improve the role it performs in the criminal justice system. Focusing on the neuroscience and social-psychology research on implicit biases and how decision making in criminal justice-related work based on these biases can be dangerous, unproductive, and unjust should begin in the forensic science classroom and reinforced during training and professional development. The legitimacy of the criminal justice system requires unbiased judgement and is necessary to earn the trust of the communities served.

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**Unconscious Bias, Forensic Education, Professional Training and Development**

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## **E134 Facing the Field: An Innovative Virtual Reality Training for Recoveries in Weapon-Contaminated Environments**

*A. Skylar Joseph, MS\*, International Committee of the Red Cross, Geneva, Switzerland; Xuan Wei, MA, International Committee of the Red Cross, Geneva, Switzerland; Ryan Yates, BSc, International Committee of the Red Cross, Geneva, Switzerland; Daniel Perkins, MEng, Remeody Plan Spatial Limited, Truro, England, United Kingdom; Pierre Guyomarch, PhD, International Committee of the Red Cross, Geneva, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will have gained awareness of the benefits that Virtual Reality (VR) training scenarios can have in forensic contexts, with particular attention toward learning to work in weapon-contaminated environments.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the need for more collaborative training approaches to tackle complex real-world situations and the benefits that Virtual Reality (VR) trainings can bring toward achieving this goal.

In recent decades, forensic expertise has become increasingly recognized as an important part of the humanitarian response to mass fatality events. When forensic expertise is needed in the aftermath of a conflict, forensic specialists and first responders will often be required to perform their work in environments that contain explosive hazards. In these situations, while maintaining dignified treatment for the dead is still a priority, the safety of the staff involved is paramount and humanitarian operations should only take place if staff are aware of the risks and know how to operate safely in a weapon-contaminated environment.

Forensic specialists, mine action staff, structural engineers, and/or emergency first responders may each need to be involved in the recovery of deceased individuals from weapon-contaminated environments. Proper training that promotes the collaboration between these different disciplines is thus necessary in order to ensure a safe, efficient, and respectful recovery. Standard training methods often require significant time and resources to organize, and it is often difficult or impossible to replicate the actual working environmental conditions one could experience. However, with the advent of VR, one can become completely immersed in realistic training scenarios.

VR simulations provide the participants with the unique experience of feeling like they are fully inhabiting and interacting with a real-world context through the creation of fully immersive 3D computer-generated environments. Due to their immersive effect, these scenarios help to not only build the cognitive and practical skills necessary for the role, but also aid in psychologically preparing the practitioner for the types of situations they may find themselves in when working in the field. Furthermore, these VR simulations aid in the standardization process for the administration of these types of trainings, acting as a beneficial supplement to courses and sensitization programs developed for varied backgrounds and levels of expertise.

The International Committee of the Red Cross (ICRC) Forensic Unit in collaboration with the Virtual Reality Unit and other units at the ICRC, including the Weapons Contaminated Unit, have been developing multiple VR training scenarios related to the proper management of the dead, including material focused on recoveries from weapon-contaminated environments. Through an overview of this scenario, its predicted benefits to the trainees, how it will promote a more collaborative working environment, and a discussion on the types of knowledge that are the most suitable to VR simulations, this presentation will seek to raise awareness of the potential benefits that VR training can have on the greater forensic community.

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### **Management of the Dead, Humanitarian Forensic Action, Virtual Reality Training Simulations**

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## **F1 Post-Conviction DNA Testing: Annual Summary of a Law School and University Collaboration to Identify and Evaluate Post-Conviction Cases**

*Katherine A. Roberts, PhD\**, School of Criminal Justice and Criminalistics, California State University, Los Angeles, Los Angeles, CA and California Forensic Science Institute, Los Angeles, CA; *Paula M. Mitchell, JD*, Loyola Law School, Los Angeles, CA; *Nikki Herst-Cook, JD*, Loyola Law School, Los Angeles, CA; *Paige McGrail, JD*, Loyola Law School, Los Angeles, CA; *Mehul Anjaria, MS, MBA* DNA Consulting, LLC, Angeles, CA

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**Learning Overview:** After attending this presentation, attendees will understand the California Forensic Science Institute-Loyola Project for the Innocent (CFSI-LPI) case review model, emphasizing case prioritization, filing and arguing motions requesting DNA testing, and strategic approaches to case resolution.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with a law school-university collaboration model to tackle the logistical challenges related to post-conviction DNA testing.

The CFSI within the School of Criminal Justice and Criminalistics at California State University, Los Angeles, established a partnership with Loyola Law School's LPI in 2019 to review cases and locate and test evidence related to violent felony offenses where actual innocence might be demonstrated. The CFSI-LPI team was awarded Department of Justice (Bureau of Justice Assistance) funding under the 2020 Postconviction Testing of DNA Evidence solicitation to provide critical assistance to individuals convicted of a serious felony that meets specific criteria. Collectively, the CFSI-LPI team reviews violent felony cases to locate biological evidence for submission to a forensic laboratory for DNA testing to demonstrate their innocence or assist in their exoneration.

This presentation provides a one-year overview of the CFSI-LPI case review model, emphasizing identifying and evaluating cases that meet the criteria under CA Penal Code §1405 for post-conviction DNA testing. This summary will include the number of DNA cases reviewed and investigated; the crimes for which clients were convicted, including those that have a sexual assault component and/or resulted in the death of the victim(s); and the number of evidence searches conducted, specifying the cases where evidence was located vs. destroyed or missing. Further, this summary will disclose the number of items submitted for Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR), mitochondrial DNA (mtDNA) analysis, and whether a viable profile was obtained in addition to the probative value of the DNA profile (supporting vs. refuting the post-conviction claim or inconclusive). Also specified will be the number of DNA profiles uploaded to the Combined DNA Index System (CODIS) vs. the number of CODIS hits. This presentation will conclude by describing obstacles to the project objectives during the 2020–2021 award period and demonstrate how the CFSI-LPI model provides forensic science graduate students an opportunity to participate in a service-learning social justice project.

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**Post-Conviction DNA Testing , Law School-University Collaboration , Service-Learning Social Justice Program**

## F2 *Qui Custodiet Ipsos Custodes?* (Who Guards the Guards?) Obligations and Rights of Parents and Children

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**Learning Overview:** To combat vaccination hesitation and encourage the acceptance of vaccination by minors, it is necessary to intervene by any means to disseminate and make available information through different communication tools on social networks and in schools.

**Impact Statement:** Adequate information is essential to ensure a reasoned choice. In fact, it is essential to provide the child with all the cognitive tools for joining the vaccination campaign, in the interest not only of their health, but of the health of those close to them and, more generally, of public health.

The World Health Organization has designated vaccination hesitation as one of the top ten threats to global health.

The vaccination campaigns against Sars-CoV-2 initially concerned the adult population, with different priorities concerning age. Following the authorization of the main agencies, including the European Medicines Agency (EMA), to combat the spread of the infection in younger people and to reduce the circulation of the virus, the Vaccination Plan also involved the population aged 12-18.<sup>1</sup>

Unfortunately, the activity of anti-vaccine movements (no-vax), also supported by the circulation of “fake news” in social networks, has created problems relating to the vaccination of minors placed in family contexts in which one and both parents are “no vax.”<sup>2</sup>

Currently, a legally authorized representative (usually a parent or guardian) must give permission (also called consent) for vaccination for someone 1217 years of age, such as by completing a written consent form that the minor (the person under the age of 18) can bring to their vaccination appointment.

In the absence of a specific law, the National Bioethics Committee unanimously, on July 29, 2021, expressed its opinion that minors between the ages of 12 and 17 may, independently, decide whether or not to undergo vaccination, regardless of the wishes of the parents and/or guardian. This intervention of the Bioethics Committee, although it has no legal value, is authoritatively part of the debate on the administration of vaccines to minors.

In the evaluation of the child’s “best interest,” it should be considered that the refusal of vaccination by the parent or guardian, in addition to the inevitable increase in the circulation of the virus, risks creating a social distance, because the parent is led to keep the child in the house to avoid social contact, as a source of possible contagion.<sup>3,4</sup>

The reduction of social contacts, particularly at very sensitive ages such as adolescence, has been correlated with the increase in the incidence of psychiatric diseases, a further reason to believe that the need to extend vaccination against SARS-Cov-2 must provide for public accountability.<sup>5</sup>

Lawmakers could enact laws that allow the child to decide on the vaccination plan, even without the consent of the parent or guardian, without the need to having to resort to the advice of the judge protect.

Adequate information is necessary to combat cases in which there is a willingness on the part of the parent or legal guardian to have the child vaccinated, but the child is opposed. The expression of the will of the child, following what is expressed by the National Bioethics Committee, cannot be accepted only in cases of consent to vaccination.

In conclusion, to combat vaccination hesitation and encourage the acceptance of vaccination by minors, it is necessary to intervene by any means to disseminate and make available information, through different communication tools, on social networks, and in schools.

For this reason, it is essential to provide the child with all the cognitive tools for joining the vaccination campaign, in the interest not only of his health, but of the health of those close to him and, more generally, of public health.

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### Sars-Cov-2, Vaccination, Social Responsibility

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## F3 Mandatory Vaccination for COVID-19? The Italian Case: Prevention Obligations and Ethical Profiles

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**Learning Overview:** After attending this presentation, attendees will understand the importance of improving knowledge about mandatory vaccinations, including for the COVID-19 pandemic.

**Impact Statement:** This presentation will impact the forensic scientific community by highlighting the importance of the topic of vaccinations. The focus on this topic has increased exponentially since the first vaccines for the COVID-19 epidemic were produced.

To be effective in eliminating communicable diseases, vaccines must be given to a sufficient number of people in the community.

Due to the success and mandatory nature of vaccinations, most people would probably not consider vaccination an optional method of medical treatment. Since the smallpox vaccine was introduced, vaccination has had its critics. In the two centuries since then, many different kinds of objections have been raised, with some questioning the scientific qualifications of mass immunization. Others have focused on the interests of personal freedom at stake and objected to the paternalistic nature of the government's imposition of what is considered a personal medical choice. Still others opposed the vaccination for personal or religious reasons.

Italy is the first nation to have imposed the obligation to vaccinate against COVID-19. The rule as it is currently limited in its operation to the deadline of December 31, 2021, and limited to a specific sector of work, health, determines important repercussions on the employment relationship for those who do not intend to accept the vaccination obligation.

With Art. 4 of the D.L. April 1, 2021, n. 44 (Urgent measures for the containment of the COVID-19 epidemic, in the matter of anti-SARS-CoV-2 vaccinations, justice and public competitions), in fact, the Government has introduced the “anti-COVID” vaccination obligation for health personnel, in order to protect public health and maintain adequate safety conditions in the provision of care and assistance services. The compulsory vaccination has also been foreseen through forms of indirect coercion, such as the reduction of the salary for those who do not want to be vaccinated and carrying out certain activities (e.g., teachers in public schools). In this context, the legislation on the so-called “green pass,” even if justified for reasons of collective health, seems to be in contrast with the provisions of the Constitution of the Italian Republic, which provides for compulsory health care only in the cases provided for by law. The Privacy Guarantor intervened on this point and with provision of May 13, 2021, n. 198, Guidance document—Vaccination in the workplace: general indications for the processing of personal data (Privacy Code—EU Regulation 2016/679).

The aforementioned legislation must be systematically interpreted with the National Strategic Vaccine Plan for the prevention of SARS-CoV-2 infections approved by Decree March 12, 2021, in which Article 1 provides that: “pursuant to art. 1, paragraph 457 of the law of 30 December 2020, n. 178, the National Strategic Vaccine Plan for the prevention of SARS-CoV-2 infections was adopted, consisting of the document ‘Elements of preparation of the vaccination strategy,’ referred to in the decree of the Minister of Health January 2, 2021.”

Paragraph 8 of Legislative Decree no. 44/2021 states that “the employer assigns the worker, where possible, to tasks, even lower, different [...], with the treatment corresponding to the tasks performed, and which, in any case, do not involve risks of spreading the infection. When the assignment to different duties is not possible, for the period of suspension [...], the salary, emolument, however called ‘is not due.’ ” The legal precept, in addition to the issues of legitimacy and constitutionality, creates a macroscopic disparity of consequences among health sector operators.

### Mandatory Vaccination, COVID-19 Pandemic, Mass Immunization

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## F4 Standards Development Activities in Forensic Science: Legal Impacts Moving Forward

*Christopher Plourd, JD\*, Superior Court, El Centro, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards within the forensic sciences.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of standards development and their legal impact. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation’s use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

The legal system—criminal, civil, and administrative—must resolve controversies. Forensic science has long been at the forefront in answering complicated questions brought before the bar of justice. Forensic scientific standards, simply stated, enhance the value and usefulness of forensic science to the legal community. A good forensic science standard not only helps assure high-quality results in the laboratory, but also shows how work performed in accordance with the standard that is both well-grounded in theory and data and that it is presented within the boundaries of “the knowledge and experience of [the expert’s] discipline.” (*Kumho Tire Co. v. Carmichael*, 526 U.S. 137, 148 [1999] [quoting *Daubert v. Merrell Dow Pharms, Inc.*, 509 U.S. 579, 592 [1993]]). Standards address four principal features that are important to the legal systems utility of a given Standard: (1) standards are written as clearly as possible, and without undefined technical terms and symbols, to enable lawyers and judges to grasp the main ideas and requirements; (2) standards describe in detail how the peer-reviewed and readily available scientific literature establishes the validity of the assumptions underlying the scientific tests and the interpretation of test results, (3) standards list the limitations of the tests and results and provide for expressions of the uncertainties in measurements and inferences drawn from them; and (4) standards include recommendations or requirements for the creation and retention of documentation of the test and the contents of reports, including the scientific limitations of the tests and related conclusions or inferences.

Forensic scientific standards developed by practitioners working with other stakeholders address all the questions being asked by courts. Courts are concerned with both technical merit and legal importance. Courts are not able to assess the scientific merit of a standard on their own. Therefore, having a process that reviews whether a Standard makes a case for the validity of the method and legal utility of the kinds of expert opinions that a standard contemplates is invaluable to the decision-making truth seeking function of the court.

During this presentation, updates related to standards development in forensic science will be presented and their legal impact will be discussed. Strategies for implementation and acceptance within the legal community will be addressed. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### Forensic Science, Standards, Legal

## F5 Strengthening Forensic Science Through Independent Verification and Validation: The Path Forward

*Marc Canellas, JD, PhD\*, Office of the Public Defender for Arlington County & the City of Falls Church, Arlington, VA and IEEE-USA AI Policy Committee, Washington, DC*

**Learning Overview:** Attendees will learn about the meaning, processes, and value of Independent Verification and Validation (IV&V) for the safety and security of our technological society and how forensic science must adopt it to ensure the evidence they generate is reliable and ensure confidence in their evidence.<sup>1</sup>

**Impact Statement:** Modern technology is impeding the ability of the forensic science community to continue its pursuit of justice through truth and evidence. These technologies like probabilistic DNA software or acoustic gunshot detection are complicated and prone to failure and manipulation in new and novel ways.<sup>2,3</sup> When they fail, innocent people are punished and accused persons are never brought to justice. When they fail, society loses confidence in the legal system and the forensic science community. This presentation will show that adopting the principles of IV&V is the best path forward for the forensic science community to ensure the evidence they generate is reliable and ensure confidence in their evidence.

“Is this the right product to correctly build for its intended use?” That is the fundamental question of IV&V, a foundational process to ensure the safety and security of our technological society from the Department of Defense’s nuclear weapons systems, the Nuclear Regulatory Commission’s regulations of nuclear power plants, to the National Aeronautics and Space Administration’s (NASA’s) manned space systems, and the Food and Drug Administration’s (FDA’s) regulation of medical systems. The Institute of Electrical and Electronics Engineers (IEEE) Standard 1012 is the world’s gold standard of IV&V and developed by IEEE Standards Association, a world-leading standards-setting organization.<sup>4</sup> IEEE 1012 codifies the requirements for independent technical, managerial, and financial verification and validation for any system likely to cause major injury, loss of safety or security, and to ensure reliability of some of the most complex and safety-critical systems in the world, including at the Department of Defense and NASA.

Despite IV&V and IEEE 1012’s wide acceptance in the science and engineering community, it has not been adopted by the forensic science community when generating evidence. New and novel hardware and software is being deployed all the time, from probabilistic genotyping (DNA) software, breath alcohol measurement devices, and face recognition, to malware detection, license plate readers, and acoustic gunshot detection. Too little time and resources are spent asking whether these systems are the right system built the right way for the intended use. As a result, systems are later found to be biased, flawed, used in improper ways, vulnerable to manipulation by users or attacks from the outside, which undermines confidence in the legal system and the forensic science community.<sup>1-3</sup> For the forensic science community to meet its pursuit of justice through truth and evidence, the community must require IV&V.

In reviewing high-profile federal cases regarding DNA software, there are several truths that must be understood in order to apply IV&V properly.<sup>5-8</sup> First, all high-risk software must comply with IV&V and evidence used to determine someone’s rights and liberties is clearly high-risk. Second, guidance documents are not standards. Standards, like IEEE 1012, are formal specifications and procedures governed by federal law and required by the Supreme Court to be produced through fair processes and without any conflicts of interest. Third, internal validation is not IV&V. IV&V requires technical, managerial, and financial independence such that none of the tools, people, organizations, or money used to perform the verification and validation have an interest in the outcome. Fourth, reliability requires objective evidence. IV&V requires systems to be evaluated based on studies, outcomes, and statistics, not merely counts of allegedly peer-reviewed articles or of how many political agencies or laboratories adopted the technique.

Ensuring the reliability of evidence used in the legal system is an awesome responsibility. It is one that will be under increasing threat due to the complexities of modern technology. For generations, IV&V has been the foundational process for engineers and scientists to meet their responsibilities for high-risk technology. The forensic science community must do the same.

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### Independent Verification and Validation, Standards, Practices and Procedures

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## F6 Giving the Defense Bar a Seat at the Table: The Importance of Ensuring a Defense Perspective in Standard Development in the Firearms Identification Field

*Kyla Wells, JD\*, The Legal Aid Society, Bronx, NY*

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**Learning Overview:** This presentation will offer attendees information about how the Firearms and Toolmarks Consensus Body functions, what steps defense attorneys should take if they are interested in participating in a consensus body, as well as emphasize the importance of the defense bar’s perspective being reflected in standard development.

**Impact Statement:** After attending this presentation, attendees will understand that the lack of uniform standards in the firearms identification field is a contributing factor to the misapplication of this forensic discipline. Attendees will also understand that a willingness by not only practitioners, but also defense attorneys, to participate in establishing standards in the firearms identification field is essential to improve the discipline and thereby prevent wrongful convictions stemming from the admission of faulty forensic evidence in courts.

**Hypothesis:** The foundational validity of firearm and toolmark examination—a subjective pattern-matching discipline that lacks an adequate empirical basis—has recently come under increasing scrutiny. A major source of criticism is the lack of uniform standards in the field. In 2009, the National Research Council issued a Report, *Strengthening Forensic Science: A Path Forward*, which emphasized the need to regulate the forensic sciences. The Report concluded:

“...Standards and best practices create a professional environment that allows organizations and professions to create quality systems, policies, and procedures and maintain autonomy from vested interest groups. Standards ensure desirable characteristics of services and techniques such as quality, reliability, efficiency, and consistency among practitioners.”

To address not only the lack of standards in the firearms identification field, but across forensic science disciplines, the National Institute of Standards and Technology (NIST) created the Organization of Scientific Area Committees for Forensic Science (OSAC) to develop much-needed standards. OSAC drafts proposed standards, sends them to standards developing organizations, who then further develop and publish the standards. The American Academy of Forensic Science (AAFS) Standards Board (ASB) is an organization consisting of consensus bodies that work closely with OSAC and its subcommittees in standard development. Members of the Firearms and Toolmarks Consensus Body of the ASB have traditionally been police officers, prosecutors, and firearms examiners. However, several defense attorneys have joined to bring a necessary defense perspective. Since joining, the consensus body has worked on developing a variety of standards aimed at moving the discipline forward, such as establishing minimum education requirements for firearms examiners. Through our participation, defense attorneys can serve as a voice for our clients who have long been the victims of faulty forensic “science.”

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**ASB, Consensus Body, Firearms/Toolmarks**



## F7 Sniffing Out Crime?: The Science and the Law of Drug Detection Dogs

*Dana Delger, JD\*, Stockholm, Sweden; Michelle Grant, Neighborhood Defender Service of Harlem, New York, NY*

**Learning Overview:** After attending this presentation, attendees will have an understanding of the basic science and law of drug detection dogs. Attendees will also learn a basic framework for undertaking a challenge to a forensic technique they have not previously encountered.

**Impact Statement:** This presentation will impact the forensic science community by explaining the current state of the science and law of drug detection dogs, as well as providing a practical understanding of how lawyers should approach this evidence.

There is little question that some animals have superior scenting abilities to humans, and human beings have relied on those abilities for thousands of years, from the truffle hogs of the Roman empire to today's COVID-19 "detection dogs." But just how superior those abilities are, and under what circumstances they can be relied upon (and for which purposes) is a more fraught question, and one that has significant consequence when they are relied on in criminal proceedings.

One such use is that of drug detection dogs, dogs routinely employed by federal and state police authorities with the ostensible ability to reliably and accurately detect the odor of illegal drugs. But available empirical evidence suggests that drug dogs' actual performances fall well short of such claims.<sup>1</sup> This presentation will cover both the science that proponents of drug dog usage point to and where that science falls short, with a particular focus on how such evidence relates to the error rate paradigm put forward by the 2016 President's Council of Advisors on Science and Technology Report.<sup>2</sup>

Drug dogs may not only be inaccurate but also biased, as research shows their results can be influenced by handler knowledge.<sup>3</sup> There is also data showing that drug dogs results may be even more biased when they are engaging with Black, Indigenous, and People Of Color (BIPOC) individuals.<sup>4</sup> This presentation will cover these issues, contextualizing them against the history of police usage of dogs against particularly Black people.

Courts have largely failed to grapple with these shortcomings, and despite its common use and the many scientific and legal issues it raises, drug dogs remain novel evidence to many attorneys. In addition to covering these failures, this presentation will also look at the evidence from the perspective of an attorney facing a first-time challenge. It will provide a basic framework for how to think through and approach a challenge that would benefit from application to any forensic science.

### Reference(s):

- <sup>1.</sup> *United States v. Bentley*, 795 F.3d 630, 636 (7th Cir. 2015) (noting that field-accuracy rates as low as 43% have been sufficient for courts to accept dogs as reliable, and finding a dog reliable despite a training policy the Court stated "seem[ed] like a terrible way to promote accurate detection").
- <sup>2.</sup> President's Council of Advisors on Science and Technology, *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods* (2016)
- <sup>3.</sup> L. Lit et al. *Handler beliefs affect scent detection dog outcome. Anim. Cogn.* 2011; 14(3): 387–394.
- <sup>4.</sup> Dan Hinkel; Joe Mahr. *Tribune Analysis: Drug-Sniffing Dogs in Traffic Stops Often Wrong. Chi. Trib.* (Jan. 6, 2011).

### Drug Dogs, Litigation, Cognitive Bias

## F8 Current Scientific Issues for Human Remains Detection Dog Cases in Court: Animal Remains and Residual Odor

Mary Cablk, PhD\*, Desert Research Institute, Reno, NV

**Learning Overview:** After attending this presentation, attendees will be aware of two components pertaining to the validity of Human Remains Detection (HRD) dog evidence being debated in United States courts at present and have a better understanding of the science pertaining to those aspects.

**Impact Statement:** This presentation will impact the forensic science community in two ways. It will inform the community of contemporary challenges to HRD dogs evidence in court and will inform anyone involved in using HRD dogs as an investigative tool of issues of reliability that may occur and may be later challenged.

HRD canines are commonly deployed to locate human remains evidence in criminal investigations throughout the United States. While the Academy Standards Board is in the process of developing a national certification standard for HRD canines, the skill and reliability of HRD teams varies widely. Regardless of certification, each canine requires assessment for reliability, and the defense has the right to review the records and challenge the training on a canine.<sup>1</sup> The prosecution relies on the HRD dog team's reliability established through certification and training.

Much of the case law pertaining to HRD dogs derives from narcotics and trailing cases, although HRD canine evidence is increasingly called into question in court in the United States. Canine final responses that produce human remains evidence such as an intact decedent, blood, bones, or DNA are generally not an issue in terms of defensibility. When an HRD canine performs a final response and no human remains evidence is located through other forensic processing means, that final response itself may be considered evidence and increasingly is presented in court as such. Here lies the question of reliability, whether the final response is a false positive or is a true positive that cannot be validated via other means.

One challenge to canine evidence in the absence of human remains is the possibility of confusion between human and animal remains, as argued in *Colorado v Redwine*.<sup>2</sup> The handler's documentation would be critical to establish the degree to which the canine had been challenged with differentiating animal and human remains, but there are still valid challenges to be had. The counter argument comes from the science, to include studies that have addressed the Volatile Organic Compounds (VOCs) that emanate from animal remains which an expert witness might use in part of the basis of their opinion on the reliability of a canine.<sup>3-5</sup>

The other challenge is the likelihood that a final response could be to residual odor of human remains, which would explain the lack of validating evidence using other methods. The issue of residual odor has been argued in numerous cases.<sup>6,7</sup> Few scientific studies have examined residual odor and canine responses to it leaving the scientific community virtually helpless to weigh in at present beyond establishing that residual odor exists.<sup>8,9</sup>

### Reference(s):

1. *Florida v. Harris*, 568 U.S. 237 (2013).
2. *People of the State of Colorado v. Mark Allen Redwine*, 2021 (District Court, LaPlata County CO Case No. 17CR343).
3. Cablk, M.E.; E.E. Szelagowski; J.C. Sagebiel. 2012. Characterization of the volatile organic compounds present in the headspace of decomposing animal remains, and compared with human remains. *Forensic Science International*. 220:118-125.
4. Rosier, E.; S. Loix; W. Develter; W. Van de Voorde; E. Cuyper; J. Tytgat. 2017. Differentiation between decomposed remains of human origin and bigger mammals. *Journal of Forensic and Legal Medicine*. 50:28-35.
5. DeGreeff L.E.; Furton, K.G. 2011. Collection and identification of human remains volatiles by non-contact, dynamic airflow sampling and SPME-GC/MS using various sorbent materials. *Analytical and Bioanalytical Chemistry* 401: 1295-1307.
6. *People v. Lang*, 862 N.W.2d 446, 308 Mich.App. 38.
7. *Commonwealth of VA v. Braulio Marcello Castillo*, 2016 (Loudoun county circuit court 26450-00, -01, -02).
8. Buis, Rebecca; Rust, Latara; Nizio, Katie; Rai, Tapan; Stuart, Barbara; Forbes, Shari. (2019). Investigating the sensitivity of cadaver-detection dogs to aged, diluted decomposition fluid. *Journal of Forensic Identification*. 69. 367.
9. Oesterhelweg; L.S. Kröber; K. Rottmann; J. Willhöft; C. Braun; N. Thies; K. Püschel; J. Silkenath; A. Gehl. 2008. Cadaver dogs—A study on detection of contaminated carpet squares. *Forensic Science International*. 174:35-39.

### Human Remains, Dog, Odor

## F9 Firearms Tool Mark Comparison: The 2008/2009 NAS Report, the 2016 PCAST Report, and the AMES Study

John Nixon, MBA\*, ARC, Bippus, IN

**Learning Overview:** This presentation is aimed primarily at jurists who wish to gain an understanding of the challenges and current state of acceptance of the firearms tool mark comparison discipline (often referred to as ballistics). This presentation focuses on the criticisms levelled at the discipline over the past two decades, and the defensive responses from the community of technicians who pay their bills by practicing this discipline.

**Impact Statement:** Attendees will gain an understanding of critical issues relating to the validity of the discipline of firearms tool mark comparison.

For millennia, humans have invested a tremendous amount of intellectual and physical resources into inventing and developing more effective ways to kill game animals, dangerous predators, and one another. Most game animals can outrun humans, so the development of personal weapons concentrated on launching projectiles that could “outrun” those game animals. When it came to warfare, being able to defeat an enemy with a projectile that had more range and accuracy than that of the opponent was a critical advantage. Projectiles have evolved over time, but essentially comprise sharp spears or arrows, and blunt trauma weapons such as pebbles and small rocks launched from hands and slingshots. The range of these weapons was limited by user strength, and the accuracy by user skill. Over the past couple of centuries, the firearm has emerged as the dominant weapon—it harnesses chemical energy to vastly increase range and, more recently, combined the laws of physics with metallurgy and manufacturing technology advances to launch spinning projectiles that provide excellent accuracy at those extended ranges. Humans now throw metal pebbles over long distances with great accuracy.

Rifles and pistols launch a single projectile that is spin-stabilized to improve accuracy and reduce drag. Most firearms spin the projectile by having a number of helical grooves cut into the interior of the barrel. The longstanding assumption has been that those machined barrels always have machine tool marks, wear defects, and corrosion that will leave discernibly unique (assumed) marks on the bullets pushed through them. Additionally, the cartridge case (typically brass) will collect discernibly unique (assumed) markings from other firearm components, such as the firing pin and the breech face.

Prosecutors have long proffered the discipline in criminal litigation and it had been accepted without challenge by the courts, defense counsel, and jurors. The proposition that all firearms left discernibly unique markings on ammunition components that were discharged in them was accepted as obvious, just as scientists had accepted as obvious, for many centuries, that the Earth was flat. Like the flat Earth theory, the concept of low error rate discernible uniqueness in firearms tool mark comparisons has been unravelling for some years, despite a few diehard proponents.

In the 21<sup>st</sup> century, astute attorneys began to challenge these assumptions, and with the aid of independent members of the scientific community, reviews were commissioned and challenges mounted. The 2008 and 2009 National Academies of Science Reports were very critical of the discipline, and their publication prompted a wave of “validation studies” by the community of practitioners.<sup>1,2</sup> The 2016 President’s Council of Advisors on Science and Technology (PCAST) Report cast doubt on the validity of the validation studies, and in the 2017 Addendum to that Report, the PCAST Committee opined that there was only one valid validation study—the AMES study.<sup>3-5</sup> The PCAST committee did note, however, that the AMES study had not been peer reviewed prior to their nod of approval. Subsequent critical review of the AMES study revealed that it was not valid on many levels, and it has been dismissed by courts. The reported false positive error rates of around 1% was too low by a massive margin – inconclusive (“I don’t know”) answers were counted as correct answers—if they are considered incorrect then the false positive error rate increases to 36%. Further, if the 23% of volunteer participants who failed to return their answers are counted as incorrect, then the false positive error rate increases to 50%. More recent studies (one claiming an error rate of zero percent) have also been discredited.

### Reference(s):

1. National Academies of Science. *Ballistic Imaging*. Committee to Assess the Feasibility, Accuracy, and Technical Capability of a National Ballistics Database. National Research Council of the National Academies, Washington DC, 2008. 322pp. (National Academies Press, March, 2008). ISBN 978-0-309-11724-1 (paperback). ISBN 978-0-309-11725-8 (PDF).
2. National Academies of Science. *Strengthening Forensic Science in the United States: A Path Forward*. National Research Council of the National Academies, Washington DC, 2009. 328pp. (National Academies Press, 18 February, 2009). ISBN-13: 978-0-309-13135-3 (hardcover). ISBN-13: 978-0-309-13131-5 (paperback).
3. Report to the President. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. Executive Office of the President. President’s Council of Advisors on Science and Technology (PCAST), 160pp, Sept 2016.
4. Report to the President. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. Executive Office of the President. President’s Council of Advisors on Science and Technology (PCAST), Addendum, 9pp, 6 January 2017.
5. David P. Baldwin; Stanley J. Bajic; Max Morris; Daniel Zamzow. *A Study of False-positive and False-negative Error Rates in Cartridge Case Comparisons*. Ames Laboratory, USDOE, Technical Report #IS-5207 (2014), at <https://afte.org/uploads/documents/swggun-false-positive-false-negative-usdoe.pdf>.

### Firearm Toolmark Comparison, 2009 NAS Report, 2016 PCAST Report

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## F10 Mixtures and Baskerville Data—The Importance of What Isn’t There

*Charles Brenner, PhD\*, DNA-VIEW, Oakland, CA and Human Rights Center, UC Berkeley, Berkeley, CA*

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**Learning Overview:** Incorporating “Baskerville data”—sub-threshold yet unarguably reliable—can turn otherwise strongly inculpatory mixture evidence on its head.

**Impact Statement:** Evaluation and calculation of possible dropout must be revised to consider Baskerville data.

“Baskerville” refers to the Sherlock Holmes story in which lack of evidence—a dog doing nothing—was evidence. Analysis of a shell casing DNA mixture was presented in a criminal case. The analysis, using a dropout-capable mixture program in the way the vendor recommends, calculated a hefty likelihood ratio of 825 connecting the defendant—call him Baltimore—to the casing. But consideration of the sub-threshold pattern of the data led to two new insights and a likelihood ratio favoring the defendant.

First, the logical reason that sub-threshold signals are unreliable data does not apply to the *absence* of a signal. It is a traditional tenet that the DNA data to be considered for evaluating the evidentiary strength of a DNA mixture should be limited to signals with height above a pre-determined “analytic” threshold rfu value. The motive behind the analytic threshold is to avoid subjectivity and bias in distinguishing real DNA signals from artifacts. The analytic threshold is by definition a level above which data is reliable. Therefore, sub-threshold signals are potentially unreliable, so accepting them risks framing (i.e., manufacturing evidence against) an innocent suspect. But that concern applies only to “data” in the positive sense of a visible signal. Negative data—information that there is no signal—unambiguously and reliably says there is no real signal.

A second, complementary point is that the *lack* of sub-threshold signals (Baskerville data) can be powerful evidence to *free* the accused as it is in the Baltimore case. The issue is dropout or, more precisely, it is computing whether dropout is a good explanation for the observed mixture data. In Baltimore’s case, the answer is “yes” when the Baskerville information is ignored, but “no” under an analysis of the real and complete data. The necessary modification to the dropout calculation is not complicated.

The issue of Baskerville data arises with all mixture software that purports to deal with dropout and that filters the data with a threshold. That includes virtually all of the available continuous and semi-continuous model programs.

An occasionally stated dictum is the wisdom that while sub-threshold information should not be used as inculpatory evidence, it can be used to exclude. How that might be done was never explained until now.

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### DNA Mixture, Sub-Threshold, Exonerate

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## F11 A Forensic Analysis of “Gang Cop” Testimony

*Sarah Sanger, JD\*, Sanger, Swysen & Dunkle, Santa Barbara, CA*

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**Learning Overview:** After attending this presentation, attendees will be aware of how to prepare for or challenge "gang cop" testimony based on forensic science principles.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of the need to meet basic forensic science (social science) standards for gang testimony.

Testimony about gangs, which can result in substantial sentencing enhancements, often is delivered by “gang cops” who derive their “expertise” from their time on the job. Sociologists have studied gangs and gang behavior, collecting empirical data and analyzing it according to scientific standards and practices. This presentation examines the scientific validity or lack thereof in “gang cop” testimony.

First, the nature and quality of the empirical data that is used to form the basis for expert opinions does not meet social science standards and practices. Observations of police officers, while interesting, are not neutral. As observers, gang cops are armed combatants who, for the most part, observe complex sociological interrelationships of members of a community from the outside. Even if they interact part of the time with the community in a constructive manner, for instance in community workshops, they are still biased observers. The data will be further distorted by the observers’ bias as members of the prosecution team.

Some efforts to curb the most significant abuses of “gang cop” testimony have been made, but do not address the core systemic problems. For instance, in California, the state Supreme Court in the *Sanchez* case, disallowed a “gang cop” witness from testifying to case-specific facts unless the witness had admissible firsthand knowledge of the facts. Typically, a witness would refer to hearsay information in police reports, Field Interview (FI) cards, or photos that were in the gang “data base.” Prior to *Sanchez*, this testimony would be presented to establish that a particular defendant was a member or associated with a gang.

Excluding this testimony in the *Sanchez* case was a step forward but only addressed part of the issue of the reliability of the data relied upon and introduced through the “gang cop” witness. Data that is not fact-specific to the defendant is still data accumulated by a police officer who claims knowledge of what is happening in a community and within alleged gangs in that community by virtue of work as a police officer. The ability to acquire unbiased knowledge of such facts is significantly impaired.

Second, opinions proffered by the “gang cop” go to the ultimate issues in the case. “Gang cops” generally testify that a defendant is a member of or associated with specific gang, that the gang is involved in criminal activity and, whatever the defendant is alleged to have done in the case at hand, was for the benefit of the gang. Like other expert witnesses, the claim of expertise and title—that of police officer—may carry undue weight in the deliberations of jurors. Yet, the standards usually associated with establishing a foundation for an expert opinion and establishing the scope of that opinion are not applied.

This presentation will conclude by exploring appropriate forensic limitations on “gang cop” expert witness testimony. One potential resolution is to prohibit the testimony as not meeting the foundational standards for an appropriate database from which to derive opinions. Another potential resolution is to prohibit such opinions even if the data could be more objectively curated, by prohibiting expert opinions as being outside of the expertise of such witnesses. This could lead to the elimination of “gang cop” experts. Some jurisdictions have found that “gang cops,” while not qualified as experts, could offer a lay opinion. That re-categorizes the problem, but does not solve it. Neither expert nor lay opinions should be permitted where they are primarily advocacy. Expert opinions on gang issues, whether or not the witness has police experience, should be based on robust empirical data and scientific standards and practices.

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### Gang, Expert, Sociology

## F12 Fire Investigation Experts: Recommendations for Expressing Opinions in Reports and Testimony

Terry-Dawn Hewitt, LLM\*, McKenna Hewitt, Denver, CO

**Learning Overview:** After attending this presentation, attendees will know the issues to be addressed in developing guidelines on how fire investigation experts should express their opinions in reports or testimony. Some of these issues may be informative to experts in other forensic disciplines that rely on the human interpretation and the scientific method to analyze evidence and form opinions.

**Impact Statement:** This presentation will impact the forensic science community by delivering practical recommendations that have been compiled from a range of studies and publications to provide guidance on how fire investigation experts are best able to express their opinions in a manner that adequately informs fact-finders without exaggerating (even unintentionally) the nature or the certainty of their opinions.

Among the significant findings of the 2009 National Research Council/National Academy of Sciences (NRC/NAS) Report are the critical questions raised about: (1) the extent that particular forensic science disciplines are based on a valid and reliable scientific methodology; and (2) the manner in which forensic science experts express their opinions in reports and in testimony.<sup>1</sup> These issues of scientific reliability and the fair and accurate reporting of opinions are inherently intertwined. After all, how can an expert express certainty in an opinion that is based on a methodology not proven to be valid and reliable? Is it necessary in the interests of justice for an opinion to address limitations in the methodologies employed, the sources of error, or possible bias? In the wake of developments triggered by the NRC/NAS Report, this presentation highlights ways to meet or surpass the challenges of rendering fair and accurate fire investigation expert opinions.

This research was broad, spanning the following sources: (1) the NRC/NAS Report, which recommended that terminology and minimum requirements for information used “in reporting and testifying about the results of forensic science investigations be standardized;” (2) the two main standards for fire investigations, the National Fire Protection Association (NFPA) 921 Guide for Fire and Explosion Investigations, and NFPA 1033 Standard for Professional Qualifications for Fire Investigator; (3) the history of debates recorded during the evolution of NFPA 921 about levels of confidence in experts’ opinions; (4) the work of the National Center for Forensic Science (NCFS) and the Organization of Scientific Area Committees (OSAC) concerning reporting and testimony; (5) the American Association for the Advancement of Science (AAAS) Quality and Gap Analyses for fire investigations; and (6) authoritative publications from other forensic science disciplines dealing with expert opinions.<sup>2-5</sup>

There are limits to the relevance of information from other disciplines. For example, the way opinions are expressed by experts in fields where findings can be reduced to statistical probabilities will not apply to fire investigations. However, there are commonalities that allow lessons to be drawn from other disciplines, such as issues of how experts express their opinions and what lay persons (non-experts such as attorneys, judges, and jurors) understand are largely matters of human communication and psychology. Like other fields, there is also a common need to identify and communicate sources of error in analyses. This presentation will summarize the results of this study, providing some practical guidance on how experts in the fire investigation field should express their opinions, communicate their level of confidence, and address limitations or sources of uncertainty.

### Reference(s):

1. Comm. on Identifying the Needs of the Forensic Sci. Cmty. et al. Nat’l Research Council of the Nat’l Acads. *Strengthening Forensic Science in the United States: A Path Forward*. (2009), available at <https://www.ncjrs.gov/pdffiles1/nij/grants/228091.pdf> [hereinafter NRC/NAS Report].
2. NRC/NAS Report, supra note 1, at 21-22.
3. Nat’l Fire Prot. Ass’n Technical Comm. on Fire Investigations. *NFPA 921 Guide for Fire and Explosion Investigations* [hereinafter NFPA 921] (1992 ed., 1995 ed., 1998 ed., 2001 ed., 2004 ed., 2008 ed., 2011 ed., 2014 ed., 2021 ed.). We use the short form, “NFPA 921” when referring generally to that document, in whatever edition is applicable in the context. When referring to a specific edition of *NFPA 921*, the edition is also cited.
4. Nat’l Fire Prot. Ass’n Technical Comm. on Fire Investigator Professional Qualifications, *NFPA 1033 Standard for Professional Qualifications for Fire Investigator* [hereinafter NFPA 1033] (2022 ed.).
5. José Almirall et al. *Forensic Science Assessments. A Quality and Gap Analysis*. Fire Investigation & Annotated Bibliography (2017), available at <https://www.aaas.org/report/fire-investigation>.

### Expert Opinion, NFPA 921, Fire Investigation

## F13 The History of Bitemark Evidence in America

*Christopher Plourd, JD\*, Superior Court of the State of California, County of Imperial, El Centro, CA*

**Learning Overview:** After attending this presentation, attendees will understand that judicial acceptance of a forensic technique does not equate to scientific validity.

**Impact Statement:** This presentation will educate forensic science practitioners and all persons in the legal community as to the future viability of forensic bitemark identification.

Forensic odontology is a branch of forensic science that has its main application in the identification of human remains. However, and less commonly, it has been used in bitemark analysis—namely, the determination about whether a particular defendant made a bitemark. This presentation will focus on the history of forensic odontology as it pertains to bitemark analysis in America. Bitemark analysis in the American judicial system dates back more than 300 years and its validity has, increasingly, become called into question. Post-conviction DNA exonerations over the past two decades have led to inquiries about the scientific validity of bitemark analysis and whether it has a legitimate place in forensic science and in the courtroom.

This presentation will examine the origination, historical development, and acceptance of bitemark evidence in the American judicial system. It will identify and discuss key bitemark cases with an examination of the arguments both for and against the use of such evidence in criminal cases. This presentation will also evaluate the turbulent history of the use of bitemark evidence and, like all lessons that experience teaches, give some predictions as to the viability of the future use of bitemarks as an accepted forensic science discipline.

Bitemark evidence was first used in the American judicial system in 1692. The Reverend George Burroughs was accused of soliciting young women into witchcraft. A significant part of the evidence used to convict him was evidence that he bit his victims. Witchcraft judges readily accepted the presentation of bitemark evidence to substantiate these allegations. Rev. Burroughs was hanged on August 19, 1692. Two decades later, Burroughs was declared innocent, and the colony of Massachusetts compensated his children for their father's wrongful execution. The Burroughs case was the first reported conviction in America based on bitemark identification. Ironically, the Burroughs case was also the first case in America of a bitemark identification exoneration.

Nearly three hundred years later, in 1954, the first bitemark identification case to be reported in an American judicial opinion was the *Texas case of Doyle v. State*.<sup>1</sup> However, it was not until 1979 during the state of Florida trial of serial killer Ted Bundy that forensic bitemark analysis gained national attention. The Bundy case was the functional equivalent of the moon landing for bitemark evidence—a turning point for forensic odontology, marking its acceptance as a legitimate forensic discipline. Over the next 30 years, numerous convictions involving bitemark evidence further cemented bitemark evidence into the legal system as admissible evidence. Some of these same cases, however, were later shown to be erroneous.

In 2009, the National Academy of Sciences (NAS) issued a Report on forensic science raising questions relating to bite mark testimony in a conclusive match."<sup>2</sup>

In 2016, the Texas Forensic Science Commission recommended a moratorium on the admission of bite mark testimony

Additionally, in 2017, the National Institute of Standards and Technology (NIST) was asked to do a scientific review and issue reports on the validity of DNA mixture, ballistic evidence, and bitemark evidence. The NIST report on the scientific validity bitemark evidence is due to be published by the end of 2021. This presentation will evaluate the report, the basis for its conclusions, and the impact it will have on the use of bitemark evidence in the American judicial system.

### Reference(s):

<sup>1</sup>. *Doyle v. State* (1954) Tex.Cr.R. 310, 263 S.W.2d 779.

<sup>2</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>.

### Odontology, Bitemark, Validity

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### F14 Y-Chromosomal Short Tandem Repeat (Y-STR) Testing in Indian Country: The Injustice of Ignoring Y-Chromosomal Variation Within and Among Tribal Communities

*J.D. Schmid, JD\*, Minnesota Board of Public Defense, Duluth, MN*

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**Learning Overview:** After attending this session, attendees will appreciate how Y-chromosomal variation within and among localized Native American subpopulations impacts the statistical weight of a Y-STR haplotype match when the suspect's presumed paternal ancestry is Native American and the relevant events occurred on or near an Indian Reservation. Practitioners engaged in Y-STR testing will be encouraged to avoid using pooled, nationwide data to assign statistical weight when the circumstances described above are present. Researchers will be encouraged to develop and validate regional databases that accurately reflect the Y chromosomal genetic characteristics of Native American subpopulations located in different geographic areas of the United States.

**Impact Statement:** This presentation will impact the forensic community by bringing awareness to the potential injustice of using a pooled, nationwide Native American database to assign statistical weight to a Y-STR match. It is by now axiomatic that any database used to assign statistical weight to a DNA match must accurately represent the population of potential contributors. A statistical calculation derived from a database that is not representative of the population of potential contributors can mislead stakeholders by exaggerating the significance of a match between a suspect profile and an evidentiary profile.

A pooled database of Native American men living in different geographic regions of the United States does not accurately represent the Y-chromosomal genetic characteristics of localized Native American subpopulations. Population research has observed a high degree of Y-chromosomal variation among Native American men depending on where the samples were taken from geographically. It has also shown that common haplotypes occur much more frequently within localized Native American subpopulations than within subpopulations of the other major ethnic groups in the United States. These observations suggest that match probabilities are, in fact, much higher within localized Native American subpopulations than would be reflected in a nationwide, pooled database.

DNA evidence is so powerful that it can undermine the ends of justice when its significance is not properly understood. Jurors, judges, and attorneys often misinterpret statistical evidence by overestimating the probative value of a match between a suspect sample and an evidentiary sample. Ignoring Y-chromosomal variation within and among Native American subpopulations compounds the risk that jurors will overestimate the significance of a match and incorrectly conclude that the suspect is the source of DNA in an evidentiary sample even when he is not. The increased risk of source misidentification will, in turn, increase the risk that innocent Native American men charged with offenses on or near Indian Reservations will be wrongfully convicted, while the real perpetrators remain in the community.

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#### Y-STR Testing, Native American Subpopulations, Statistical Weight



## F15 Understanding Juror Comprehension of Forensic Testimony: Assessing Jurors' Decision Making and Evidence Evaluation

Cassidy Koolmees BS, BA\*, Florida International University, Miami, FL; Devon LaBat, BS, Florida International University, Miami, FL; Jacqueline Evans, PhD, Florida International University, Miami, FL; Nadja Schreiber Compo, PhD, Florida International University, Miami, FL; Deborah Goldfarb, JD, PhD, Florida International University, Miami, FL

**Learning Overview:** Attendees will be provided with information about juror decision-making in cases involving forensic print evidence.

**Impact Statement:** The current research will provide insight into how jurors perceive forensic evidence testimonial quality and whether jurors need additional support to assess testimonial quality.

Testimony by forensic scientists can have a powerful impact on jurors' perceptions of the guilt or innocence of the accused. At the same time, forensic evidence often relies on highly scientific and technical methods that jurors may have difficulty comprehending. Research suggests that putative jurors struggle with understanding the statistics and technical language used to convey forensic science.<sup>1</sup> Furthermore, jurors often do not interpret forensic evidence in the way that expert examiners intend, such that they can misperceive the strength of the evidence by relying on factors external to the evidence.<sup>2,3</sup> Although previous work has explored how the content and language used by forensic experts can impact juror decision making, no known study has examined whether jurors are sensitive to testimonial quality.

Recently, the Department of Justice released a Uniform Language for Testimony and Reports document for forensic print examiners. This document included five guidelines that forensic print examiners should follow when creating reports and testifying as an expert witness in court. First, a forensic expert should not claim that two prints came from the same person and from no other person (or to the exclusion of all other persons). Second, a forensic expert should not state that forensic print examination is without error. Third, a forensic expert should not give a conclusion about prints that includes a statistical or numerical degree of probability except when based on relevant and appropriate data. Fourth, a forensic expert should not cite the number of forensic print examinations he or she has done in his or her career as proof of the accuracy of their conclusion in this case. And fifth, a forensic expert should not use the expressions "reasonable degree of scientific certainty," "reasonable scientific certainty," or similar claims of reasonable certainty in either reports or testimony about print evidence. The current study examined whether jurors were sensitive to testimonial quality using these guidelines and explored the impact of testimonial quality on juror decision making and perception of forensic evidence.

Participants viewed and listened to transcripts of a mock criminal trial using language from real-world criminal trials. Trial transcripts included preliminary jury instructions, brief opening statements, direct and cross-examination of witnesses for the prosecution and the defense (including a forensic print examiner), closing statements, and final instructions. Participants were randomly assigned to conditions in a 2 (case fact pattern) x 3 (forensic evidence type: fingerprint, footwear print, tire-print) x 6 (level of forensic testimonial quality) design. Participants were presented with a version of a trial transcript consistent with their randomly assigned condition. After reading and listening to the trial transcripts, all participants were asked to provide feedback as if they were a juror on the case, including: providing a verdict and rating their confidence in their verdict; rating the overall evidence on a variety of measures (e.g., strength, quality, clarity); rating the forensic science testimony on a variety of measures (e.g., strength, quality, clarity); and rating the forensic scientist (e.g., credibility, qualifications, effectiveness).

Data collection is almost complete. Preliminary results indicate that the quality of testimony has no effect on verdict decisions, perceptions of strength of the evidence overall, perceptions of the forensic scientist, or perceptions of the forensic science evidence. This suggests that mock-jurors are not sensitive to low-quality forensic testimony and need additional support to differentiate between low- and high-quality testimony. Legal and policy implications will be discussed.

### Reference(s):

1. Martire, K.A.; Kemp, R.I.; Watkins, I.; Sayle, M.A.; Newell, B.R. (2013). The expression and interpretation of uncertain forensic science evidence: Verbal equivalence, evidence strength, and the weak evidence effect. *Law and human behavior*, 37(3), 197.
2. Eldridge, H. (2019). Juror comprehension of forensic expert testimony: A literature review and gap analysis. *Forensic Science International: Synergy*, 1, 24-34.
3. Koehler, J.J.; Schweitzer, N.J.; Saks, M.J.; McQuiston, D.E. (2016). Science, technology, or the expert witness: What influences jurors' judgments about forensic science testimony? *Psychology, Public Policy, and Law*, 22(4), 401.

### Forensic Testimony, Juror Decision Making, Print Evidence

## F16 Juror Understanding of the Weight of Evidence Presented as a Likelihood Ratio and the Impact on the Deliberative Process

*Bess Stiffelman, JD\*, Law Office of Bess Stiffelman, Los Angeles, CA and Independent Juvenile Defender Program, Los Angeles, CA*

**Learning Overview:** Attendees will better understand how the lack of intuitive understanding of the weight of evidence presented as a likelihood ratio impacts the deliberative process in criminal trials, and whether this evidence should be limited or recharacterized to prevent miscarriages of justice.

**Impact Statement:** With the advent and popularity of probabilistic genotyping, software likelihood ratios are becoming an increasingly popular tool for evaluating and presenting the probative value of evidence, particularly in the context of complicated mixtures of DNA. This presentation will help explain how even though likelihood ratios may be appropriate tools when they are used to evaluate evidence in research contexts or as investigative leads, they may not be the best method for communicating the weight of the evidence in criminal trials.

Legal reasoning relies on a different set of foundational concepts than forensic science such that, although the disciplines often interact and rely on one another, they do not always share the same objectives. Although we are all concerned with outcomes and want to minimize wrong decisions while optimizing right ones, our legal system is not merely consequentialist. There are aspirational principles at play as well—symbols of respect and autonomy that lie at the heart of the criminal justice system. As articulated by William Blackstone in the 1760s, our system is founded on the principle that “it is better that ten guilty persons escape than that one innocent suffer.” The presumption of innocence and the corollary burden of proof beyond a reasonable doubt create limitations on what evidence should be heard and how it is presented. Relevant evidence can, and often does, conflict with these principles and is therefore not heard by a jury. These likelihood ratios threaten to undermine those bedrock principles.

A previous article raised some of these concerns about the use of likelihood ratios in the presentation of DNA mixture evidence interpreted using probabilistic genotyping software.<sup>1</sup> J. Buckleton et al. responded with a critique and technical corrections purporting to assuage any such concerns.<sup>2</sup> This article’s focus on the purely technical aspects, while perhaps appropriate to the discipline of forensic science, nevertheless failed to account for and grapple with the legal principles at play and the larger concern about juror understanding of the weight of evidence communicated as a likelihood ratio. This created an opportunity for a conversation between the legal community and our forensic science colleagues about the competing principles at play, and the impact on the deliberative process.

Likelihood ratios produced by probabilistic genotyping software express the probabilistic relationship between two specific hypotheses that seek to explain the observed characteristics of a given piece of DNA evidence. Although a familiar concept to many here, this is not as easy for the rest of us to understand. As one judge recently described it, probabilistic genotyping is “a combination of forensic DNA techniques, mathematical theory, statistical methods (including Monte Carlo Markov Chain modelling, as in the Monte Carlo Gambling venue), decisional theory, computer algorithms, interpretation, and subjective opinions . . .”<sup>3</sup>

Intuitively, without an understanding of the probabilistic reasoning at play, likelihood ratios appear to jurors as something that they are not. Thompson and Newman found that “[a] surprisingly high percentage of our participants (about two thirds) indicated that one or both statements consistent with the source probability error were a ‘correct interpretation’ of what the expert said. These fallacious interpretations were not merely matters of semantics—[they] found that they were strongly associated with verdicts and estimates of the probability that the defendant was guilty.”<sup>4</sup>

In order for jurors to properly understand and incorporate a likelihood ratio into their deliberations, Bayesian reasoning must be applied. This requires converting the prior assessment of guilt or innocence into a probability and thus quantifying that prior belief. It is this act of quantifying the prior that creates one of the challenges. This is not how jurors make decisions, and when likelihood ratio evidence is introduced, they are not instructed on Bayesian reasoning. As the Adams Court that considered this in the United Kingdom concluded, “to introduce Bayes Theorem, or any similar method, into a criminal trial plunges the jury into inappropriate and unnecessary realms of theory and complexity deflecting them from their proper task.”<sup>5</sup> So, we are left with the other option: incorporating the evidence in an intuitive manner, which is how this happens in most trials. This leads us right back to the concern that jurors will be unable to determine the weight of the DNA evidence presented as a likelihood ratio.

Since factfinders in a criminal trial will overvalue likelihood ratio evidence, and DNA evidence is often already given undue weight because of the “CSI effect,” this use of likelihood ratios may lead to miscarriages of justice.

### Reference(s):

1. Bess Stiffelman. No Longer the Gold Standard: Probabilistic Genotyping is Changing the Nature of DNA Evidence in Criminal Trials. *24 Berkeley J. Crim. L.* 110 (2019).
2. J. Buckleton et al. A review of likelihood ratios in forensic science based on a critique of Stiffelman “No longer the Gold standard: Probabilistic genotyping is changing the nature of DNA evidence in criminal trials.” *Forensic Sci. Int.* 310 (2020) 2, 110251.
3. *U.S. v. Gissantaner*, Western District of Michigan, Case 1:17-cr- 130 (2019).
4. W. Thompson; E. Newman. Lay understanding of forensic statistics: Evaluation of random match probabilities, likelihood ratios, and verbal equivalents. *Law and Human Behavior*, 2015.
5. Adams (1996) EWCA Crim 10 and 222.

### Likelihood Ratio, DNA Evidence , Presumption of Innocence

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## F17 The Role of Opinions, Probability, and Likelihood Ratios in Forensic Evidence Communication by Experts

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**Learning Overview:** Attendees will become familiar with the distinction between probabilities, which are numerical expressions of personal degrees of belief, and relative frequencies, which report how often an event of interest has actually occurred in real life during a given duration of time. A likelihood ratio, being a ratio of two probabilities, is also a personal assessment and is, in fact, an opinion expressed using a numerical scale. One may, incorrectly, view these personal assessments, expressed numerically, as being rigorous and objective because of the mathematical or statistical tools used to arrive at them. A proper understanding of the information being communicated by an expert, in the form of probabilities or as likelihood ratios, either in their report or during testimony, requires additional information that can help the trier of fact judge the reliability of the expert's testimony. This presentation explains these concepts in detail. No background training in probability or statistics is needed to appreciate the relevance of the information presented.

**Impact Statement:** This presentation will help the forensic science community better understand the nature of the information presented in reports and/or testimony, using a probabilistic framework, and will enable them to meaningfully evaluate the reliability of expert testimony by focusing on available supporting information rather than based on the expert's credentials alone.

Forensic science experts have the difficult task of communicating their findings, after analysis of forensic evidence, to triers of fact and other decision makers in the judicial system. Ever-increasing technical complexity resulting from scientific advances and cutting-edge practices and challenges associated with the treatment of uncertainty add to the difficulty of communicating accurately and effectively. In many forensic disciplines, experts report their findings in the form of a conclusory opinion. Many forensic scholars argue that it is inappropriate for an expert to offer conclusions, as conclusions are the domain of the decision makers and doing so may suppress meaningful consideration of factors that could lead a decision maker to reach a different conclusion. Instead, it is often argued that experts should summarize their findings by providing a likelihood ratio as the strength of the evidence. This presentation emphasizes that likelihood ratios are opinions too, and concerns related to experts providing conclusions have analogs with experts providing likelihood ratios (or other forms of probabilistic interpretation). Various issues associated with experts using probabilities and likelihood ratios to communicate forensic findings are discussed. Motivated by the notes to FRE 702, alternatives are explored that do not require experts to summarize their findings using an opinion (conclusory or probabilistic) instead focusing on the specialized knowledge that they possess by virtue of their training and experience.

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### Likelihood Ratio, Probability, Reliability

## F18 Should Low Likelihood Ratios (LRs) Be Admissible? Considering the Kinship Problem

Clinton Hughes, JD\*, Brooklyn Defender Services, Brooklyn, NY

**Learning Overview:** This presentation will demonstrate that without a factual and scientific basis for ruling out relatives in a DNA mixture, the elimination of the uninformative range and the unqualified reporting of low LRs in forensic DNA analysis threaten to run afoul of the fitness for purpose standard of *Daubert*.<sup>1</sup>

**Impact Statement:** Considering the prevalence of law enforcement searches of family homes and other examples of semi-closed environments where relatives have access, the criminal justice system should not dismiss DNA mixtures of related individuals as some kind of rare outlier when considering low inculpatory LRs.

In their 2020 research paper, *Are low LRs reliable?*, several leaders in the field of forensic DNA offered context for the 2018 SWGDAM Guideline 3.1, which advised that even low LRs should not be considered inconclusive.<sup>2,3</sup> This study contrasted a closed environment—for example, a crime scene with fewer than five possible donors—with an open environment where anyone with access to the crime scene could be a possible donor. This study claimed that in a closed environment, even low-discriminating LRs may be probative. However, this approach ignores a common and predictable scenario of a closed or semi-closed environment—a crime scene such as a family home or family car where related individuals have access. With related individuals as a common issue, it is critical to evaluate the significance of any low LR by ruling out whether a non-contributor relative of the true contributor(s) to a DNA mixture might be falsely associated with that mixture. While non-contributor testing with this common scenario has been disturbingly sparse, a few internal validations of probabilistic genotyping tools have indicated a much higher false positive rate for related non-contributors than with random unrelated profiles.<sup>4</sup> In certain situations, false positive LRs with related individuals can reach into the high quadrillions. Considering the prevalence of law enforcement searches of family homes and other examples of semi-closed environments where relatives have access, the criminal justice system should not dismiss DNA mixtures of related individuals as some kind of rare outlier when considering low inculpatory LRs. By reporting low LRs without underlying validation, labs are complicit in shifting the burden of proof to defendants to establish that the true contributors are relative[s], rather than requiring the prosecution to rule out a very common exception. This presentation will demonstrate that without a factual and scientific basis for ruling out relatives in a DNA mixture, the elimination of the uninformative range and the unqualified reporting of low LRs in forensic DNA analysis threaten to run afoul of the fitness for purpose standard of *Daubert*.<sup>1</sup>

### Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals, Inc.* 509 U.S. 579 (1993).
2. J.S. Buckleton et al. *Are low LRs reliable?* Forensic Science International: Genetics 49 (2020) 102350.
3. Validation summaries available at <https://indefenseof.us/issues/kinship-problem>; <https://epic.org/state-policy/foia/dna-software/EPIC-16-02-02-CalDOJ-FOIA-20160219-STRmix-V2.0.6-Validation-Summaries.pdf>; <https://epic.org/state-policy/foia/dna-software/EPIC-15-10-13-VA-FOIA-20151104-Production-Pt2.pdf>.
4. *Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios.* Scientific Working Group on DNA Analysis Methods (2018), available at [https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0\\_dd5221694d1448588dcd0937738c9e46.pdf](https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0_dd5221694d1448588dcd0937738c9e46.pdf).

### Likelihood Ratio, Probabilistic Genotyping, Relatedness

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## F19 Touch DNA Communication in the Courtroom: Developing Methods for the Effective Communication of Forensic Science Data

*Julie Burrill, PhD\*, Alan Alda Center for Communicating Sciencefor, Stony Brook, NY and King's College London, London, NA, United Kingdom*

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**Learning Overview:** Attendees will hear about novel research results into the content of touch DNA deposits. They will also learn about new methods for scientists to consider when communicating their forensic data to each other, to attorneys, or to juries.

**Impact Statement:** This work seeks to improve the way that scientists communicate their results and put them appropriately into context for a legal audience. Additionally, the novel touch DNA results presented here will further clarify the cellular and cell-free components as well as the human and non-human DNA content in touch deposits, which has a direct impact on their potential to transfer between people and items.

Forensic science exists to be utilized in a legal setting. Consequently, it is only as valuable as the scientists' ability to communicate their process and results in the courtroom and interpret the meaning of scientific data in context for the fact finder. This can be particularly challenging when conveying complex results with nuanced layers of interpretation such as those from DNA mixtures or low-level samples such as "touch DNA."

Research at the Alan Alda Center for Communicating Science is investigating the relative success of various methods of communicating these scientific complexities in the courtroom. This work includes analysis of existing trends in testimony and report writing and is currently developing and testing new communication strategies for both scientists and attorneys. This presentation will use touch DNA as an exemplar evidence type and explore how to best improve effective communication of novel research and/or casework results. Original research quantifying the relative contributions of cellular and acellular DNA to a potential touch deposit will be presented.

Hand rinses were collected from washed and unwashed hands. Each sample was processed and characterized using newly adapted methods for short DNA fragments. DNA was characterized with traditional Short Tandem Repeat (STR) typing as well as massively parallel sequencing to evaluate degradation patterns and microbial content. Results will be discussed in the context of the current scientific and legal landscape; potential techniques for the dissemination of these data for various target audiences will be presented. The Alda Method combines improvisational theater techniques that help scientists to pay dynamic attention to the needs of their listeners and learn to spontaneously adjust to those needs. This approach integrates techniques to help scientists distill their message, shape compelling stories, create common ground, and use conversational language that fosters genuine human connections. This presentation explores the applicability of this and related approaches to touch DNA results in a courtroom.

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**Touch DNA, Science Communication, Courtroom**

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## F20 The Application of the OSAC Registry of Standards to the Revised Federal Rules of Evidence Rule 702

*Mark Stolorow, MS, MBA\*, National Institute of Standards and Technology, Special Programs Office, Gaithersburg, MD*

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**Learning Overview:** This presentation will provide an examination of the proposed changes to Rule 702 and the relationship of implementation of the standards on the OSAC Registry to the demonstration of ensuring compliance with those changes.

**Impact Statement:** This presentation will discuss the relevance and potential impact standards implementation have on the application of the newly proposed amendment and case notes to Rule 702.

In April 2021, the federal Advisory Committee on Evidence Rules (ACER) proposed amendments to the wording of Federal Rules of Evidence Rule 702 which pertains to expert testimony. Briefly, those proposed changes encourage courts to more rigorously adhere to ensuring that “forensic expert testimony is valid, reliable, and not overstated in court.”

Since its official inception in 2014, the NIST Organization of Scientific Area Committees (OSAC) for Forensic Science has advanced the mission of strengthening forensic practice by facilitating the development of technically sound, science-based standards through a formal standard developing organization (SDO) process, by evaluating existing standards published by SDOs for placement onto the OSAC Registry, and by promoting the use of the OSAC Registry approved standards throughout the forensic science community.

How does the implementation of standards on the OSAC Registry impact conformance with the revised Rule 702? This presentation will address the implications.

With the list of OSAC approved standards growing, the community has rightfully turned its attention to the implementation of these standards into the quality systems of forensic laboratories. As of the drafting of this abstract, OSAC has posted 65 standards involving 15 different forensic science disciplines plus interdisciplinary standards. From preliminary national OSAC standards implementation survey results closing in August 2021, more than 100 forensic science service providers have already implemented standards listed on the OSAC Registry into their quality management system documents.

Since September 2020, OSAC has also implemented Scientific and Technical Review Panels (STRPs) to ensure that standards contain processes for determining and reporting measurement uncertainty whenever possible. Standards need to reference supporting scientific literature to provide experts and the courts with foundational documents supporting the validity and reliability of the principles and methods. OSAC aspires for the STRPs to ensure that standards will meet those needs.

What relevance and potential impact does standards implementation have on the application of the newly proposed amendment and case notes to Rule 702? Standards implementation and adherence to those standards in the forensic analysis, reporting and testimony can demonstrate to the courts that the proffered expert opinions are not overstated and comport with nationally approved standards grounded in valid and reliable methodology.

This presentation will provide an examination of the proposed changes to Rule 702 and the relationship of implementation of the standards on the OSAC Registry to the demonstration of ensuring compliance with those changes.

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### Standards, Federal Rules of Evidence, OSAC for Forensic Science

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## F21 Medical Biobanks—Different Aims, Same Controversies: How to Approach the Constitutional Lens to Bioethical Dilemmas of Human Rights, Civil Claims, and Solving Crimes

*Maria Ciruzzi, PhD\*, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Buenos Aires, Argentina and Law School, University of Buenos Aires, Buenos Aires, Buenos Aires, Argentina*

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**Learning Overview:** After attending this presentation, attendees will be confronted with the dilemmas and conflicts that the use of medical technology poses in different areas of human behavior, showing that there is no unambiguous answer where we can feel comfortable.

Attendees will also acknowledge the importance being open minded, exercising critical thinking, and dealing with dilemmatic situations in a non-binary world.

**Impact Statement:** This presentation will impact the forensic and social community by showing key aspects of the important role that law, ethics, and technology are called on to perform in a rapidly changing world under almost omnipotent technology.

“Genetic information” distinguishes between the genetic material itself and the genetic information: the “genetic material” will always be with the person from the first stages of life until the moment of death, while the genetic information is obtained by performing a series of techniques or methodologies that allow the extraction of certain specific data. In this sense, it is affirmed that genetic information is the set of data of genetic origin and nature that is settled on a specific support, it may be a file or a specific information bank.

It should be noted that not all genetic information is private. There is genetic information associated with phenotypic traits, that is, external traits that are public knowledge, such as eye color, height, complexion, etc. The genetic information that deserves protection is that of sensitive management and generally associated with health and predisposition to certain diseases of genetic origin and late onset. That information is private, at least until the first symptoms appear or until the individual intentionally discloses this information.<sup>1</sup>

Genetic data could certainly be very useful, but we know that not all that is technically possible as it could be bioethically grounded and/or legally binding.

Millions of human biological samples are stored worldwide for medical research or treatment purposes. These samples are also of enormous potential value to law enforcement, as DNA profiles can be obtained from these samples but for different goals.

This presentation will expose a real medical case as a trigger to compare the different views that may arise from a single biological sample when the objectives differ: medical use, parental responsibility, crimes against humanity, or the resolution of cold cases. Based on this, this presentation will try to assess the interaction between bioethical dilemmas, judicial controversies, protection of human rights, and the exercise of the state punitive power.

### Reference(s):

- <sup>1</sup> De Groot Nina F.; va Beers Britta; Decock Lieven; Meynen Gerben. Accessing medical biobanks to solve crimes: Ethical considerations. *J Med Ethics* 2021;47:502-509

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### Medical Biobanks, Human Rights, Crime Investigation

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## F22 Shifting Forensic Science Development From the Organization to the Discipline

*Claude Roux, PhD\*, Centre for Forensic Science, University of Technology Sydney, Broadway, NSW, Australia; Sheila Willis, PhD, Leverhulme Research Centre for Forensic Science, University of Dundee, Dundee, NA, United Kingdom; Celine Weyermann, PhD, Ecole des Sciences Criminelles, Université de Lausanne, Lausanne, VD, Switzerland*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of forensic science as a discipline and be able to differentiate scientific fundamentals focused on a purpose from a scientific organization focused on means and technologies.

**Impact Statement:** The presentation will promote and encourage discussions with the community, including judges, prosecutors, defense attorneys, and policy makers, on how ongoing challenges in forensic science may be best addressed by shifting the focus from the organization to the discipline purpose.

Forensic laboratories and services are a mainstay of the criminal justice system across the world. The usefulness of (forensic) science is therefore widely recognized. However, several reports indicate that forensic science usefulness in court remains very limited and is occasionally highly controversial. Different problems have been identified, such as justice miscarriage, backlogs, unreliable scientific process, inadequate training, lack of reference data, etc. However, attempts to solve these issues (i.e., more funding, quality management, bias mitigation, statistics, shared databases) do not seem to have durably solved the forensic science “crisis.” It is argued that some solutions may even have brought further unforeseen problems. For example, distancing scientists from the crime scene may forward independence and decrease bias. Still, it also increases linkage blindness (i.e., the ability to connect different pieces of information in casework, including essential information for subsequent scientific examination).

An alternative path forward is suggested in this contribution by contrasting the forensic science means and purposes under the prism of the discipline (i.e., forensic science) rather than the organization (i.e., practice). Indeed, nowadays, forensic science is mainly viewed as applying science(s) to legal matters and has barely been defined as an academic discipline (at best, it is a subdiscipline of medicine, chemistry, biology, or computer science). Thus, the forensic science development has essentially been driven by organizations (such as, but not limited to, forensic laboratories). It is argued that organizational response to the forensic crisis has focused on improving and standardization of means and processes. However, organizations and processes are highly dependent on the political, economic, and legal structures in which they operate. This may explain why proposed solutions could hardly be efficiently applied transversally to all forensic science models.

Moreover, new tools and technologies are continuously developed by a quasi-infinite number of different scientific disciplines, thus leading to further diversity and fragmentation of forensic science. On the other hand, as a distinct discipline, forensic science can refocus research and development on shared principles and purposes, such as reconstructing, monitoring, and preventing crime and security issues. Scientific research and development must mainly be carried out from within a discipline in a scientific (bottom-up) rather than political or economic (top-down) approach to be relevant and efficient.

This presentation will show how shifting the forensic science focus from means to a purpose provides a basis on which organization (s) and current practice(s) can more adequately evolve, eventually leading to a more impactful and long-lasting improvement of forensic science basic knowledge and subsequently application.

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### Relevance, Technology, Research and Development



## F23 Forensic Science Leading the Fight Against Illicit Pharmaceuticals

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**Learning Overview:** Attendees will learn about the global health threat of counterfeit pharmaceuticals and how pharmaceutical companies can provide specific expertise in authentication and identification using traditional forensic approaches to partner with law enforcement in an effort to protect public health.

**Impact Statement:** This presentation aims to build awareness of the global threat of counterfeit pharmaceuticals and how forensic principles and techniques around quality, analysis, and interpretation help support law efforts to mitigate this threat.

Counterfeit medicines and illicit pharmaceuticals are a worldwide problem that have critical public health and safety implications that continue to escalate. Collected intelligence on the subject for calendar year 2020 reported 4,344 unique pharmaceutical counterfeiting, diversion, and theft events, involving 2,451 different medicines, and spanning 137 countries. A historical review of counterfeit events shows a significant increase in counterfeit events involving oncology medicines.<sup>1</sup>

The incredible complexity required to authenticate pharmaceutical products highlights the need for brand owners to play a role. Expertise for a company's entire product portfolio that encompasses physical printing and packaging characterization, chemical characterization, unknown compound identification, and even proteomics is crucial. In addition to the analytical requirements, to be able to support legal action, those analytical needs must be paired with the forensic science legal standards of strict chain of custody and evidence management controls, **International Organization for Standardization (ISO)** accreditation standards required for evidentiary data submissions, and expert witness training to testify as subject matter experts.

Merck® has designed and built a global laboratory network that meets these demands and is dedicated to the detection and characterization of counterfeit and illicit medicines. Partnered with health organizations, government agencies, and law enforcement departments, these ISO 17025-accredited labs test for product authenticity and characterize counterfeit or illicit products in an effort to protect patient safety.

A case study involving a counterfeit oncology product will be presented highlighting how the Merck® laboratories were able to use analytical tools and forensic techniques to characterize the counterfeit products and use those characterizations to drive actionable and defensible forensic intelligence. This intelligence developed through laboratory analyses was able to link multiple cases globally to a single organized crime group operating clandestine laboratories that managed a global distribution network of the counterfeit product. Partnerships with key investigators and law enforcement groups led to the arrests and successful prosecution of the organization's leaders and the disruption of the illegal enterprise.

From this success, the Merck® forensic laboratories look for where to go from here by looking for additional traditional forensic techniques that can help drive forensic intelligence and meaningful outcomes like the shared example. Techniques around enhanced protein analysis, stable isotopic signatures, and reverse engineering of counterfeits are beginning to be added into the repertoire in an effort to push the fight against counterfeit and illicit pharmaceuticals.

### Reference(s):

<sup>1</sup>. Pharmaceutical Security Institute, 2020 Situation Report.

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### Counterfeit, Pharmaceuticals, Analytical

## F24 Court Proceedings During the COVID-19 Pandemic

*Julianna Firek\*, Duquesne University, Pittsburgh, PA; Lyndsie N. Ferrara, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** After attending this presentation, attendees should better understand how greatly the court system has been affected by the COVID-19 pandemic.

**Impact Statement:** This presentation impacts the forensic science community by affecting the way courts are run and how testifying may look in the future

**Synopsis:** As of March 2020, the courts shut down, along with the rest of the world. During this unprecedented time, figuring out a way to continue with legal proceedings was a difficult feat that each court did independently, without universal guidelines. Prior to the pandemic, the court was extremely slow to make changes, but the pandemic forced many quick changes along with significant investment into new courtroom technology. These significant changes are important to document and analyze.

The goal of this research was to collect firsthand accounts of court adaptations and operations throughout the pandemic. Interviews were conducted with various professionals within the court system to gather information on their experiences during the pandemic. Interviews were conducted across different jurisdictions in order to compare the changes each court made. These interviews were conducted via Zoom or in person, and the responses were compared to each other and what was found in literature. This comparison identified challenges confronting the court system as well as elicited unique perspectives specific to different jurisdictions. Courtroom operations were examined in light of legal rights and requirements such as the Sixth Amendment right to a speedy and public trial.

Documenting how the courts adapted is important for future events that may require virtual court as well as for analyzing if operations were effective and fair. As the pandemic continues, court operations continue to adapt. This research provides a retrospective analysis of courtroom operations that shifted to fully virtual proceedings up through re-opening efforts and hybrid models. During the shutdown period, lengthy backlogs continued to increase. Addressing this back log will require a unique and efficient approach in order to ensure every person obtains equal access to justice.

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### COVID-19, Virtual, Court

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## F25 Perpetuating the Presumption of Guilt: The Role of Implicit Racial Bias in Forensic Testimony

*Janis Puracal JD\**, Forensic Justice Project, Portland, OR; *Aliza Kaplan, JD*, Forensic Justice Project, Portland, OR and *Lewis and Clark Law School, Portland, OR*

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**Learning Overview:** After attending this session, attendees will have a better understanding of how implicit racial bias can impact forensic opinions and how systems should be reformed to mitigate that risk.

**Impact Statement:** This presentation will impact the forensic science community by presenting the research on implicit racial bias and how that bias can contribute to wrongful convictions.

Since the murders of George Floyd and Breonna Taylor, conversations around racial bias in the criminal justice system have accelerated. Much of the focus has turned to police reform. The potential for racial bias, however, does not end with the initial stop, search, and arrest. Rather, it can be found throughout our criminal justice system, and forensic testimony is one area that does not get nearly enough attention when it comes to implicit racial bias. Lawyers, judges, and jurors often approach scientific sounding evidence with a predisposition to accept the expert’s conclusions, without a critical eye. Experts for the government are often presumed to be neutral and objective witnesses who report the science with no stake in the outcome. Government experts, however, are susceptible to bias, including implicit racial bias, just like the rest of us. These experts who testify in criminal cases may not be reporting “neutral science;” the testimony may be contaminated by implicit racial bias, among other biases, that has colored their conclusions.

Pattern-matching methods—for example, analysis of firearms and tool mark impressions, bloodstains, latent prints, hairs, and footwear and tire impressions—are especially problematic. These methods are inherently subjective, meaning that they rely heavily on the examiner’s individual judgment, rather than any objective standard. Even though the examiner may be analyzing, for example, a bullet from a crime scene, that examiner may also be given “irrelevant contextual information,” such as the name, race, and background of the lead suspect. Knowing that information can predispose the examiner to believe that the bullet was fired from that suspect’s gun, leading to a result-driven opinion about whether the marks on the bullet “match” the marks on test fires from the gun. The human eye will see what it wants to see, and, because there are no objective standards by which to measure the marks, the examiner’s conclusions may reflect bias in favor of guilt, rather than a true determination that the marks are, in fact, the same in appearance or sufficient in number.

A number of recent exonerations have raised questions about the role of implicit racial bias in faulty forensic testimony that resulted in a wrongful conviction. This presentation addresses how implicit racial bias can affect forensic testimony and ways to address it before wrongful convictions occur.

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### Implicit, Race, Bias

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## F26 The Art of Retrieving Documents From Messaging Apps

*John Carney, JD\*, Carney Forensics, Scandia, MN*

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**Learning Overview:** This presentation will briefly introduce the practice of mobile device forensics and explain why mobile apps are key sources of document evidence. It will explore six questions with attendees that go to the heart of document retrieval from mobile messaging apps based on new technology platforms:

- Which mobile devices must be examined for message and document retrieval?
- What types of messages must be recovered?
- What types of documents are to be retrieved from those messages?
- How much metadata must be collected to provide foundation and authenticate those documents?
- What about recovery of documents and messages subject to spoliation?
- What other message and document sources can be probed when mobile devices are not available?

**Impact Statement:** The presentation will impact attendees in terms of competence (ability) and performance (action) by showing the expanded definition and range of modern document evidence. It will provide practical advice and effective forensic techniques for recovering documents attached to popular mobile messages found on smart phones and other mobile platforms. It will teach attendees how to preserve the integrity and authenticate messaging and document evidence for admission into the record of federal and state courts. And, it will show credible demonstrative exhibits of document evidence competently retrieved from messaging apps on mobile device platforms to help attendees visualize successful outcomes.

This presentation will focus on effectively using mobile device forensics to recover new and traditional forms of document evidence attached to popular mobile messaging apps used by billions of people worldwide today. The proposition of this presentation is to acknowledge and come to grips with the expanding definition and role of document evidence in the modern world, including government, academia, business, and jurisprudence. This presentation centers on the need for attorneys, paralegals, and investigators to develop new processes and forensically sound techniques for document recovery and forensic collection. The objective is the satisfaction of the rules of evidence to support admissibility of material evidence from billions of smart phones and tablets upon which cases are likely to turn today. Document retrieval from messaging apps is more “art” than science for many legal practitioners in 2022. They lack a viable process capable of producing repeatable and defensible results. They mastered document recovery from emails decades ago, but the retrieval of messages and their documents on new mobile technology platforms elude much of the legal profession. A paralegal will usually start from scratch for each case or each client. They use ad hoc or case-specific methods that often disappoint and produce only screenshots or worse. Ultimately, parties are frustrated and the bench faces admissibility challenges due to forensically unsound recovery or collections.

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### Mobile Device Forensics, Messaging App Evidence, Document Retrieval

## F27 Pandemonium in Social Media: A Collision Course With the Fourth Amendment

Gary McDonald, JD\*, Dallas County Criminal District Attorney's Office, Dallas, TX

**Learning Overview:** The purpose of this presentation is to educate attendees about emerging trends in the collection of location data by social media companies.

**Impact Statement:** This presentation will impact attendees by helping them understand the Fourth Amendment implication of the collection of this information, the forensic viability, and the commercial uses for which it is collected.

Social media permeates today's electronic world. It is estimated by recent research that adults in the United State use social media in the following percentages: Facebook®—69%, Instagram™—40%, Snapchat™—25%, and Twitter®—23%.<sup>1</sup> Users of these services, by way of example, opt into Terms of Service Agreements that allow the collection of metadata and information, including location information. For instance, Facebook® and Instagram™ are governed by the same Data Policy that includes metadata, location information, usage, and device information, which may be used for a valid business purpose, as described, but may also be accessible to law enforcement in furtherance of criminal investigations.<sup>2</sup> For other social media companies such as Google®, it has been reported that location information collected was an invaluable tool in the identification of those involved in the Capitol protests on January 6, 2021, and that such information is collected and stored in the Google® Sensorvault, which aggregates location information from a variety of sources.<sup>3-5</sup>

After this presentation, attendees will have a deeper appreciation of the sources and uses of such information for corporate and law enforcement purposes—especially as it relates to emerging trends for the forensic use of information, such as in geofences. As the nature and type of information collected increasingly impacts individual privacy, the Electronic Communication Privacy Act of 1986 will be compared to emerging more limited state privacy provisions.<sup>6</sup> The recently adopted Subchapter (G-1), Texas Code of Criminal Procedure and the California Electronic Communications Privacy Act both restrict law enforcement access to such prospective location information through traditional tools and provide a statutory exclusionary rule, if the information is obtained outside of the state statutory provisions.<sup>7,8</sup> This shift from federal law, which contains an exclusivity of remedies provision for information obtained through the Stored Communications Act may serve as a trend toward the limitation of the use of such information in forensic science and investigations.<sup>9</sup>

### Reference(s):

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- <sup>2.</sup> <https://www.facebook.com/about/privacy/update>.
- <sup>3.</sup> *United States v. Harvey*, 1:21-CR-00212-ABJ, United States District Court for the District of Columbia, March 8, 2021, Statement of Facts in Support of Criminal Complaint.
- <sup>4.</sup> Google's Sensorvault is a Boon for Law Enforcement. This Is How It Works. *New York Times*, New York, N.Y., (April 13, 2019). <https://www.nytimes.com/2019/04/13/technology/google-sensorvault-location-tracking.html>.
- <sup>5.</sup> Google's Sensorvault Can Tell Police Where You Have Been. *Electronic Frontier Foundation*, San Francisco, C.A., (April 8, 2019) <https://www.eff.org/deeplinks/2019/04/googles-sensorvault-can-tell-police-where-youve-been>.
- <sup>6.</sup> 18 U.S.C. §§ 2510-2523 (1986).
- <sup>7.</sup> Tex. Code Crim. P. Chapter 18B, Subchapter (G-1), Vernon Tex. Code Ann. 2021.
- <sup>8.</sup> Cal. Penal Code § 1546 (2016).
- <sup>9.</sup> Compare 18 U.S.C. 2708 with Tex. Code Crim. P. Art. 18B.553 (Vernon Tex. Code Ann. 2021), Tex. Code Crim. P. Art. 18B.326 (Vernon Tex. Code Ann. 2021), and Cal. Penal Code § 1546.4 (2016).

### Social Media, Privacy, Location Information

## F28 Picture Me Rollin’: Personal Privacy in the Land of Automated License Plate Readers

Michael Buresh, JD\*, Cook County Public Defender, Chicago, IL, USA

**Learning Overview:** This presentation will introduce attendees to the popular police mass-surveillance technology of Automated License Plate Readers (ALPRs), high-speed cameras that detect and record every license plate they encounter. While United State police departments spend millions of dollars on ALPRs, only a small fraction of their indiscriminate surveillance proves useful to public safety. Worse yet, these devices are deployed disproportionately in communities of color, leading to terrifying and potentially fatal encounters between armed police and the people of these communities. This presentation will teach attendees the basics of how these devices work, how police use them, how effective they are, and the legal implications of their use.

**Impact Statement:** This presentation will impact the forensic science community by encouraging consideration of the utility of a mass surveillance technology. Attendees will learn the costs, both financial and human, of indiscriminate surveillance of people’s public movements.

ALPRs are high-speed cameras that photograph vehicle license plates in their immediate environment. When an ALPR camera scans a license plate, the camera records an image of the plate, along with the date, time, location, and photograph of the vehicle and its plate. ALPR software then uploads these images of license plates, called “detections” or “plate scans,” to a centralized database. ALPRs can be mounted on fixed infrastructure like poles, or mounted on police or other city vehicles.

Police departments contract with third-party companies for access to these companies’ databases of ALPR detections. The most prolific ALPR service provider is Vigilant Solutions, which maintains a database called the National Vehicle Location Service (NVLS). Law enforcement agencies can create lists of wanted license plates (“hot lists”) in these databases. If a plate is in a hot list at the time it is scanned by an ALPR camera, this is called a “hit.” When a hit occurs, the system will notify the law enforcement agency whose reader recorded the hit, as well as the agency who entered the plate into the hot list. Law enforcement clients can also perform historical searches by entering a license plate number into the database and the database will return a list of all instances in which its ALPR cameras detected that plate. Law enforcement agencies can also elect to make their NVLS-hosted detections accessible to other law enforcement agencies. This presentation will present details of law enforcement agencies’ use, including statistics obtained via public records requests.

While ALPRs sometimes provide valuable intelligence to law enforcement agencies, this surveillance technology also raises serious privacy and discrimination concerns. Most obviously, these devices conduct constant, indiscriminate mass surveillance of entire communities, regardless of whether any individual is suspected of committing a crime. Further, civil liberties groups, such as the American Civil Liberties Union, the Electronic Frontier Foundation, and the Brennan Center for Justice, have raised concerns about the discriminatory impact of ALPR camera deployment.<sup>1-3</sup> In addition to exposing communities of color and other vulnerable populations to over-policing, the discriminatory deployment of ALPR cameras can also signal to those communities that they are unvalued. “Surveillance that disproportionately targets communities of color carries a distinct and cognizable equal protection harm: branding them with a badge of inferiority.”<sup>4</sup> For example, in Chicago, the city’s initial deployment of fixed-location ALPR cameras in 2017 focused primarily on Black and brown neighborhoods on the South and West sides of the city.

This presentation will also address the current legal landscape of ALPRs. While most courts that have addressed the issue have found that law enforcement’s broad surveillance via ALPRs does not violate individuals’ right against unreasonable searches and seizures, most of these decisions were issued before the United States Supreme Court’s landmark decision in *Carpenter v. United States*.<sup>5</sup> In *Carpenter*, the Court ruled that the Fourth Amendment prohibits law enforcement’s warrantless tracking of peoples’ movements via their cell phones, even when most of those movements occur in public.

### Reference(s):

1. American Civil Liberties Union. *You Are Being Tracked: How License Plate Readers Are Being Used To Record Americans’ Movements*. (2013), available at: <https://www.aclu.org/files/assets/071613-aclu-alprreport-opt-v05.pdf>.
2. Electronic Frontier Foundation. *Street Level Surveillance: Automated License Plate Readers (ALPRs)*. (2017), available at: <https://www.eff.org/pages/automated-license-plate-readers-alpr>.
3. Brennan Center for Justice. *Automatic License Plate Readers: Legal Status and Policy Recommendations for Law Enforcement Use*. (2020), available at: [https://www.brennancenter.org/our-work/research-reports/automatic-license-plate-readers-legal-status-and-policy-recommendations#footnote27\\_bxc7gmi](https://www.brennancenter.org/our-work/research-reports/automatic-license-plate-readers-legal-status-and-policy-recommendations#footnote27_bxc7gmi).
4. Brennan Center for Justice. *Automatic License Plate Readers: Legal Status and Policy Recommendations for Law Enforcement Use*. supra. *Carpenter v. United States*, 138 S. Ct. 2206 (2018).

### Surveillance, Privacy, License Plate

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## F29 Michael Williams v. Forensic Gunshot Audio Detection: How Unvalidated Forensic Evidence Sent an Innocent Man to Jail

*Brendan Max, JD\*, Cook County Public Defender, Chicago, IL*

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**Learning Overview:** Attendees will learn how police and prosecutors attempt to use unvalidated forensic gunshot audio detection evidence in criminal trials. This presentation will discuss the forensic method employed by ShotSpotter<sup>®</sup>, Inc. to detect and locate gunshot events in complex urban environments. This presentation will describe the substandard approach taken by ShotSpotter<sup>®</sup> to validate their forensic method, as well as the inaccurate claims made by ShotSpotter<sup>®</sup> regarding the performance of their forensic method. Finally, attendees will learn how such unvalidated evidence cost Michael Williams his freedom when he was charged with murder based largely on forensic evidence generated by ShotSpotter<sup>®</sup>.

**Impact Statement:** The forensic science community will gain an inside look at the forensic method employed by ShotSpotter<sup>®</sup>, a method that has undergone very little scrutiny in the forensic science community. The forensic science community will gain new insights into the inadequate attempts by ShotSpotter<sup>®</sup> to validate their method and will also gain critical knowledge of the bases relied upon by ShotSpotter<sup>®</sup> when making performance claims about their forensic method. The result of this presentation will be to spur on vital outside scrutiny of the ShotSpotter<sup>®</sup> forensic method, which has been lacking for many years.

Michael Williams was charged with murder based on evidence generated by the ShotSpotter<sup>®</sup> forensic method despite the fact that the ShotSpotter<sup>®</sup> method has never undergone meaningful scrutiny by the forensic science community and despite the fact that the method has never been adequately validated for use in criminal trials. The lack of scrutiny stems, in part, from the fact that most in the forensic science community and the criminal justice system know very little about the ShotSpotter<sup>®</sup> method and its weaknesses and limitations. While ShotSpotter<sup>®</sup> has repeatedly issued claims about the performance of their forensic method, such as a false positive rate below 1%, these claims are not scientifically derived and fail to provide an accurate assessment of method performance. This presentation will discuss the ShotSpotter<sup>®</sup> method, performance claims by ShotSpotter<sup>®</sup>, and how those claims fail to tell the true story of ShotSpotter<sup>®</sup> method performance. This presentation will describe how the ShotSpotter<sup>®</sup> forensic method has avoided scientific scrutiny despite widespread use in the criminal justice system. Through the example of the Michael Williams case, this presentation will describe how police and prosecutors used questionable ShotSpotter<sup>®</sup> evidence to charge Mr. Williams with murder and incarcerate him for nearly a year before prosecutors decided to dismiss the charges, and will further discuss how prosecutors use their discretion to avoid litigating the reliability and admissibility of evidence generated by the ShotSpotter<sup>®</sup> evidence. Finally, this presentation will call for more transparency from ShotSpotter<sup>®</sup> as well as meaningful scrutiny from the forensic science community.

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**ShotSpotter<sup>®</sup>, Gunshot, Audio**

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## F30 The Dangerous Creep of Investigative Forensic Techniques Into Criminal Trials

*M. Chris Fabricant, JD\*, Innocence Project, New York, NY*

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**Learning Overview:** The goal of this presentation is to discuss how forensic techniques such as ShotSpotter<sup>®</sup>, facial recognition technology, cadaver dog evidence, and others that were developed for investigative purposes are beginning to be introduced in court as direct evidence of guilt. More specifically, this presentation will discuss the pressure to introduce at trial forensic evidence that has led to the identity of a suspect—or to other evidence—but has not been scientifically validated, and how the use of such evidence threatens the fair administration of justice.

**Impact Statement:** This presentation will impact the forensic community by elucidating how novel—often proprietary—techniques are being pushed into court for purposes beyond the intended use of the technology and before scientific validation. For example, ShotSpotter<sup>®</sup> was developed to direct police to a geographical area to investigate potential gunshots. Courts, however, have admitted ShotSpotter<sup>®</sup> evidence to prove gunshots were, in fact, fired. As more and more criminal investigations rely on forensics to develop suspects and/or corroborate prosecution theories, there is corresponding pressure to allow juries to hear all the evidence that led to the arrest/indictment. Courts typically make admissibility rulings based on the facts of the particular case and not on how such rulings influence other courts. A single *Daubert* or *Frye* decision can open the floodgates to the unfettered use of technology that is not sufficiently reliable to make life and liberty decisions. Moreover, and equally concerning, unvalidated technologies are being used to justify a level of police intrusion (e.g., providing the basis for a warrant, or creating, in the case of facial recognition, an unduly suggestive identification procedure that becomes the basis for an arrest). In such cases, courts must allow the accused to confront and interrogate these techniques, even when they lurk in the background because they *create* the more traditional evidence that then ends up in court.

DNA exonerations over the past 30 years have demonstrated the unacceptable risk of the introduction of unvalidated and unreliable forensic sciences in criminal trials. Evidence that began as investigative techniques, such as bitemark evidence, were accepted by one court in one case, leading to widespread acceptance by courts across the country—and over 30 wrongful convictions and indictments.<sup>1</sup> Over the past decade, there has been a proliferation of proprietary forensic techniques marketed to law enforcement agencies as infallible. As these techniques come online, there is inevitable pressure by law enforcement to allow juries to hear evidence that may or may not be reliable. Moreover, expert witnesses in these emerging areas often have a financial interest in courts' acceptance of the method. As society becomes ever more dependent—and credulous—of technology, courts must exercise their gatekeeping responsibilities to reject evidence that has not been proven reliable, whether this evidence is the product of the technologies themselves or was obtained using the unreliable technologies and, as such, is similarly suspect. The failure to do so will lead to the same pattern of blind acceptance of unreliable forensics that has resulted wrongful convictions. This presentation will be an opportunity to learn and discuss how to prevent this cycle from recurring.

### Reference(s):

- <sup>1</sup> M. Chris Fabricant; W. Tucker Carrington. The Shifted Paradigm: Forensic Science's Overdue Evolution from Magic to Law, 4 *V.A.J. CRIM. L.* 1, 38 (2016)(documenting the first bite mark case in human skin, *People v. Marx*, 54 Cal. App. 3d 100 (2d Dist. 1975), and its influence on jurisprudence nationwide).

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Digital Technology, *Frye/Daubert*, ShotSpotter<sup>®</sup>



## F31 A Strict Metrological Approach to the Validity as Applied of DNA Profiling

Veronica Scotti, JD\* Politecnico di Milano, Milano, MI, Italy; Alessandro Ferrero, MSc, Politecnico di Milano, Milano, MI, Italy

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the different uncertainty contributions that may lead to doubts about the reliability of DNA profile extraction and how they should be estimated to assess the validity as applied in each specific case.

**Impact Statement:** This presentation will impact the forensic science community by presenting a scientifically sound method for estimating the probability that possible errors in the obtained DNA profiles may lead to incorrect decisions.

Validity and reliability of DNA profiling methods have been discussed since the very first application of this method in the forensic field and the discussion has never stopped, mainly because of the number of miscarriages of justice originated by incorrect interpretations of the obtained profiles.<sup>1</sup>

This discussion has mainly considered the random match probability and its correct evaluation (especially when cold hits are considered) as the main parameter for assessing the validity of DNA profiling methods.<sup>1</sup> However, it was also proven that the presence of false positive errors may significantly affect the maximum likelihood ratio used to weigh the plausibility of the inclusion hypothesis with respect to the exclusion one.<sup>2</sup>

Several contributions are available aimed at evaluating the probability of false positive errors, but they are mainly based on the analysis of Quality Assurance (QA) issues detected by the forensic labs.<sup>3-5</sup> While these are interesting contributions, showing that errors are possible, they cannot be applied to each single DNA evaluation and do not consider errors that could not be identified by the QA system. This approach does also neglect two important points:

Most steps in DNA profile extraction require accurate measurement procedures directly related to the material under test (DNA quantification, fluorescence, etc.) or to influence quantities (such as temperature), and measurement uncertainty may affect the final result.

There is a growing request for forensic labs to be accredited according to **International Organization for Standardization (ISO) 17025** standard, that mandatorily requires uncertainty evaluation.<sup>6</sup>

This presentation will discuss these points and how the evaluation of all uncertainty contributions, including those coming from the analysis of QA issues, is expected to provide a reliable estimate of the probability of wrong identification in the specific considered case. Reference to the Amanda Knox case is also considered, since exoneration was based on similar considerations.

### Reference(s):

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3. Kloosterman, A.; Sjerps, M.; Quak, A. Error rates in forensic DNA analysis: Definition, numbers, impact and communication. *Forensic Science International: Genetics* 12, pp. 77–85 (2014).
4. Anderson, J.F.; Martin, P.; Carracedo, A.; Dobosz, M.; Eriksen, B., et al. Report on the third EDNAP collaborative STR exercise. *Forensic Sci. Int.*, 78, pp. 83–93 (1996).
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6. ILAC Modules in a Forensic Science Process, ILAC-G19:08/2014 (2014). *ILAC*.

### Forensic Metrology, DNA Profiling, Validity as Applied

## F32 Making Something Out of Nothing: The Inconclusive Fallacy

*Kelley Kulick, JD, Santa Clara County Public Defender's Office, San Jose, CA; Mark Perlin, PhD, MD\*, Cybergenetics, Pittsburgh, PA*

**Learning Overview:** After attending this presentation, attendees will understand the significance of an “inconclusive” forensic result. They will learn how to prevent the unethical misuse of inconclusive results, promoting relevant and reliable forensic evidence in the courtroom.

**Impact Statement:** This presentation will impact the forensic community by educating both lawyers and scientists about the ethical considerations and serious risks inherent in the admission of inconclusive DNA results into evidence.

“You can’t get something for nothing”—First Law of Thermodynamics

“ $0+0=0$ ”—Immediate from the Peano Postulates of Natural Numbers

“Nothing will come of nothing”—King Lear, Act I, William Shakespeare

It is well known in science, mathematics, and literature that one cannot create something out of nothing. Adding together an infinite number of zeros still sums to zero. But in forensic science, some lawyers argue that the absence of forensic evidence implies guilt. That “inconclusive” DNA evidence means that the defendant is “included” in DNA evidence. This fallacious reasoning is wrong and dangerous.

The argument goes like this. First, a forensic expert reports that some evidence (DNA, for example) is “inconclusive” for a defendant. Then, an attorney has their expert agree that, based on these “inconclusive” results, the defendant “cannot be excluded” from the DNA evidence. The lawyer goes on to tell the jury that since the defendant cannot be excluded, he “must be included” in the DNA evidence and, therefore, is guilty as charged.

This logical fallacy misapplies the “law of the excluded middle.” The lawyer is incorrectly saying that the defendant must either be “excluded” or “included” from the evidence; there is no other possibility. But there is a third possibility—the evidence is “inconclusive”—and so no “excluded” or “included” determination can be made.

Expressed as a Likelihood Ratio (LR), when an LR match statistic equals one, there is no support in the evidence for a hypothesis (e.g., defendant inclusion) or its alternative (exclusion). Scientists use the logarithm of the LR to express information. The log of 1 is 0. So, there is zero information about inclusion or exclusion. Zero is not a positive  $\log(LR)$  supporting inclusion, nor is zero a negative  $\log(LR)$  supporting exclusion. A zero  $\log(LR)$  means zero information.

Many crime laboratories apply thresholds to their statistical LR results. Even though they calculate an informative match statistic (say, an inclusionary  $LR=1,000$ , or an exclusionary  $LR=1/1,000$ ), they do not report this LR and instead call the result “inconclusive.” Better science would have them report the LR value, along with an error rate that provides context for the jury (for example, given the chance that someone who didn’t leave their DNA in the evidence would have a match statistic as strong). That helps prevent the unscientific transformation of informative DNA evidence into inconclusive results.

Arguing that “inconclusive” forensic non-results imply guilt is not admissible evidence. An “inconclusive” statement has no probative value—it has no ability to make a relevant disputed point more true or less true. Moreover, Federal Rules of Evidence (FRE) Rule 403 on relevant evidence permits courts to exclude even relevant evidence, should its probative value be substantially outweighed by a danger of unfair prejudice, confusing the issues, or misleading the jury.

This presentation introduces the “inconclusive fallacy,” where lawyers concoct something nonsensical out of nothing forensic. This presentation will illustrate the fallacy’s misuse in recent criminal cases where “inconclusive” arguments harmed justice and will discuss the ethical implications of misleading a jury through non-probative forensic evidence. Practicing trial lawyers need to be aware of this maneuver so they can raise timely objections to vacuous arguments that subvert forensic science.

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### Inconclusive, Forensic Reporting, Error Rate

## F33 The Impact of Post-Traumatic Stress Disorder (PTSD) on the Crime Scene: Expected Results

*Donatella Curtotti, PhD\**, Department of Law, Foggia, Italy; *Giovanni Messina, PhD*, Foggia, Italy; *Francesco Sessa, PhD\**, Department of Clinical and Experimental Medicine, Foggia, Italy; *Wanda Nocerino, PhD*, Department of Law, Foggia, Italy; *Fabrizio Vanaria, MD*, Department of Medical, Surgical, and Advanced Technologies, G.F. Ingrassia, Catania, Italy

**Learning Overview:** After attending this presentation, attendees will have a better understanding of a correct Crime Scene (CS) approach.

**Impact Statement:** This presentation will impact attendees in terms of competence (ability) and performance (competence put into “action”) in handling the pressure and post-traumatic stress at the CS.

Forensic sciences aim to provide answers to the questions underlying a criminal event, through the examination of the CS. Experts belonging to different forensic branches are called to gather evidence, but the identification of such evidence and how it is linked to the crime is often difficult due to the shortage of specialized personnel, the lack of suitable professional equipment, and the convulsive conditions.<sup>1</sup> In this regard, the emotional implications deriving from the examination of particularly bloody crime scenes should not be minimized.

It has been noted that in repeated activities at the CS, some operators show particular behavioral evidence, manifesting a state of Post-Traumatic Stress (PTS). Repeated exposure to this stress due to the nature of the profession can result in Post-Traumatic Stress Disorder (PTSD). In PTSD, individuals experience extreme terror, helplessness, and pain after witnessing extremely painful and traumatic cases, such as murders, acts of terrorism, and suicides of family members and colleagues, even if they are not directly exposed to harmful or threatening situations.

Stress induces the activation of the Sympathetic Nervous System (SNS) and of the Hypothalamic-Pituitary-Adrenal axis (HPA), modifying a whole series of biological parameters.<sup>2-4</sup> Consequently, the conditions of emotional stress can affect the rational abilities of the operators who intervene in various ways on the crime scene (first aid personnel, first responders, the Crime Scene Investigator [CSI] team), causing cognitive biases that can alter the logical reasoning that must always characterize intervention on the CS.

Due to the centrality of the examination of the CS in the criminal trial, the University of Foggia in collaboration with the Italian Scientific Police, is conducting research (still in the testing phase, to be finished by December 2021) to verify whether the post-traumatic emotional stress disorders of the operators approaching the CS may affect (and to what extent) the results of the investigations conducted, in terms of the efficiency of the results acquired there.

The aim of this paper is to share the preliminary results of this experiment on the stress conditions of operating personnel on CS, distinguishing between first aid personnel, first responders, and the CSI Team.

The verification process was staggered as follows:

**Administration of a questionnaire of investigative habits.** First, an online questionnaire has been distributed to a selected number of subjects belonging to different professional associations engaged in CSI.

**Collection of information on personal experiences of stress and anxiety on the CS.** Participants are asked to report on the state of stress, anxiety, and coping mechanisms. To measure stress levels, the Perceived Stress Scale (PSS), the Police Stress Questionnaire (PSQ), and the Distress Thermometer will be used.

**Questionnaire elaboration and evaluation of the impact of PTSD on the CS.** The following parameters shall be assessed at this stage: emotional intelligence; death anxiety; and PTS. The following dosages are performed at time 0 (time of enlistment), time 1 (after three months), and time 2 (six months), correlating them with the events the subject has witnessed.

**Results of the experiment and possible drafting of a standardized operational protocol.** According to the response of the experimentation, solutions will be advanced to contain the risk of investigative errors caused by emotional stress. In particular, it will evaluate the opportunity of resorting to the introduction of Standard Operating Procedures (SOPs). The provision of adequately detailed and meticulous standardized procedures could neutralize the dangers deriving from the errors of reasoning of CS operators caused by personal traumatic experiences.

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### Crime Scene, Post-Traumatic Stress Disorder, Standard Operating Procedures (SOPs)

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## F34 Flawed Forensic Evidence in South Korea's Wrongful Convictions

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**Learning Overview:** This study examines the flawed forensic evidence that contributes to wrongful convictions in South Korea. In the eighth case of the Hwa-seong Serial Murders cases, which was retried in 2020, errors in the application of neutron activation analysis and hair microscopic analysis were finally confirmed. In the Nakdong-Riverside Murder Case, which was retried in the same year, it was confirmed that the judge misinterpreted a number of forensic evidence, including the suspect's argument for his visual impairment. In other retrial cases, errors in estimating the time of death and misinterpretation for bloodstain analysis were revealed. In addition, in the course of the retrial, prosecutors usually submitted unverified forensic evidence in order to maintain their guilt conviction.

**Impact Statement:** In recent years, wrongful conviction cases have appeared in the news media and as they are also covered in popular movies and dramas, public interest in wrongful convictions is growing considerably. However, the limitations are clear. Most just pay attention to the individual case, and vibrant academic research on wrongful conviction is still lacking. In most cases, false confession is considered as the primary cause of injustice, and there is insufficient interest for the other causes of wrongful conviction. However, as research by American scholars has shown, flawed forensic eyewitness misidentification and jailhouse informants are also problematic.

This study examines the errors of forensic science evidence revealed in the wrongful conviction cases in South Korea and compares them with foreign cases.

In fact, for a long time, criminal justice professionals in South Korea did not seriously recognize the possibility of errors in criminal adjudication. The conviction rate in criminal trials has been maintained at close to 99%, which is very similar to the case of Japan, well known for its high conviction rate. Most of the miscarriage of justice that have been revealed so far has been in political or ideological cases, involving false confessions due to torture, assault, or manipulation of evidence.

Obvious errors in general criminal cases were extremely rare, and some cases of wrongful convictions were even considered very exceptional cases. However, changes are now taking place. This is because under the influence of the global Innocence movement, efforts to uncover errors in criminal trials have become increasingly active. In particular, in recent years, wrongful conviction cases have appeared in the news media and, as they are also covered in popular movies and dramas, public interest in wrongful convictions is growing considerably.

However, the limitations are clear. Most simply pay attention to an individual case, and vibrant academic research on wrongful conviction is still lacking. In most cases, false confession is considered the primary cause of injustice, and there is insufficient interest for the other causes of wrongful conviction. However, as research by American scholars has shown, flawed forensics, eyewitness misidentification, and jailhouse informants are also problematic.

This research compares the Korean experience with the status quo of American cases.

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### Flawed Forensic Evidence, South Korea, Wrongful Conviction

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## F35 When DNA Lies: One Family’s Reckoning With an Erroneous Test Result

*Melanie Armstrong, BA\*, ThroughLine Consultants, Vienna, VA; Tynan Peterson, MA, ThroughLine Consultants, Oakland, CA*

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**Learning Overview:** After attending this presentation, attendees will better understand the power of genetic genealogy and its use in a new context.

**Impact Statement:** This presentation will impact attendees by sharing a landmark case in which genetic genealogy found the truth despite previous scientific evidence to the contrary.

Genetic genealogy combines autosomal DNA data from at-home DNA tests with traditional genealogical research to determine the level and type of genetic and familial relationship between individuals. Most often used to help identify an adoptee’s likely birth parents or the unknown parent of a child of a Non-Paternity Event (NPE), genetic genealogy has also relatively recently been used as an effective crime-fighting tool—helping to identify criminals, exonerate the innocent, and give names to victims of violent crimes, also known as Jane and John Does. While genetic genealogy has been used for years, the rise in popularity of consumer DNA tests continues to increase the effectiveness of this approach. There are more databases and more samples in those databases, making searches easier. They are more popular globally, enabling cross-border and international searches. This explosion of data broadens the range of situations in which genetic genealogy can uncover the truth. The uses of forensic genetic genealogy—genetic genealogy in a legal context—are continuing to evolve. Fertility fraud cases—where fertility doctors have impregnated their patients under false pretenses—are on the rise. In addition, instances of rape and incest—and their perpetrators who fathered children—are being identified for the first time.

The unique case study presented in this session will show how genetic genealogy was used in yet another legal context. Twenty years ago, a custody battle was settled based on the results of a paternity test. A family was torn apart and the child’s parentage a complete mystery. Eighteen years later, a genetic genealogy analysis correctly identified the birth father and proved that the long-ago paternity test was wrong. A new paternity test confirmed these results and revealed how the first test was mishandled by the original lab. The family was divided because of human error. In one of the first cases of its kind, the impacted family is pursuing legal action against the two companies responsible for the original test, suing both for negligence “in obtaining, labeling, handling, testing, and reporting tests results.” The case is working its way through the legal process.

The implications of this case are vast: credibility and accountability of DNA labs, irreparable damage to families, and legal precedence, to name a few. In this case, there is a strong possibility that another family also received inaccurate results and is still unaware of the mistake. DNA doesn’t lie, but to err is human. Genetic genealogy is a powerful investigative tool that can bring the truth to light.

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### Genetic Genealogy, Forensic Genetic Genealogy, DNA Testing

## F36 Cannabis Green Tongue: Anecdotal Science and Legal Fiction

Gerald Feigin, MD\*, Gloucester Medical Examiner Office, Sewell, NJ; Gil Sapir, JD, MSc, Chicago, IL

**Learning Overview:** After attending this presentation, attendees will understand that a “green tongue” is a toxicological and legal fiction for cannabis use.

**Impact Statement:** The forensic science community will be impacted through anecdotal green tongue’s non-compliance with scientific standards for evidential use.

Police officers use the purported “objective” observation of a dorsal “green tongue,” “green coating,” “green film,” and “green tint” as one of several signs to justify probable cause for arrests in Driving Under the Influence of alcohol or Drugs (DUI/DUID).<sup>1</sup> However, there is no supporting scientific evidence, case law, or authority for it. The “green tongue syndrome” is more of an interest and misuse of police science than actual toxicology. A review of relevant literature provides an anecdotal basis for a green tongue from smoking cannabis.<sup>2</sup>

Probable cause is commonly defined as: A reasonable ground to suspect that a person has committed, or is committing a crime, or that a place contains specific items connected with a crime.<sup>3</sup> Being a marijuana user, by itself, is not reasonable suspicion or probable cause.

A green tongue is caused by food coloring, including FD&C green No.3 or a mix of Blue 1 or Blue 2, Yellow 5, and Food and Drug Administration (FDA)-approved grape skin extract or disease.<sup>4,5</sup> The most common disease is thrush, a fungal infection, caused by the yeast *Candida albicans* with a minimal faint green color.<sup>3</sup>

The green component in marijuana is chlorophyll. Chlorophyll is denatured by heat at 60°C and, therefore, cannot stain a tongue green.<sup>6</sup> The chemical and physical composition of cannabis smoke does not contain chlorophyll.<sup>7</sup> Tobacco and cannabis do not color the tongue when smoking cigarettes or vaping.<sup>8</sup> Smoking marijuana will not cause a green tongue.

Law enforcement, Drug Recognition Experts (DRE), and the National Highway Traffic Safety Administration (NHTSA) primarily rely on dated, unreliable, and misquoted articles taken out of context, without supporting authority, to infuse credibility for a subjective cannabis determination. Furthermore, cited literature omits chlorophyll heat degradation. The current February, 2018 Drug Recognition Expert Course manual states, “possible green coating on the tongue” and “a greenish coating on the tongue has been documented in two peer-review articles.”<sup>9-11</sup> Proponents of green tongue identification often employ numerous qualifiers throughout related articles without definitive empirical data, thereby subjecting the content to speculation, conjecture, and unreliability.<sup>12,13</sup>

DREs through law enforcement and NHTSA are serial anecdotalists. DREs testify that “outward signs that somebody may be under the influence of [marijuana]” include (1) “raised taste buds,” (2) “a green stripe down their tongue,” that “looks like a dull green film on their tongue” and results from “marijuana debris and film in their mouth.”<sup>14</sup>

“ ‘Junk science’ is the mirror image of real science, with much of the same form but none of the same substance.”<sup>15</sup> Junk science or pseudo-science routinely consists of flawed, unreliable, exaggerated, and sometimes fabricated testimony usually founded on questionable methodology.

Law enforcement, NHTSA, and DREs are purveyors of green tongue fiction and irreparable harm. The scientific accuracy and legal reliability of a green tongue for cannabis use is unsubstantiated in current scientific literature. Green film tongue syndrome should be considered pseudoscience or junk science. It cannot be employed or referenced with any aspect of DRE training and law enforcement use. Accordingly, a green film tongue is not a reasonable suspicion or probable cause for a determination of cannabis use.

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2. Blewitt, Dennis L. Gonzo ravings: Real and Imaginary Dangers, House of Lies - all to convict and collect money, (NHTSA Study Question), Feb. 9, 2014, containing e-mail correspondence from Barry K. Logan to Jay Watterworth, Oct. 27, 2010 concerning green tongue, “At best I would say the information appears to be anecdotal.” <http://gonzo2ravings.blogspot.com/2014/02/real-and-imaginary-dangerous.html> (accessed Aug. 20, 2021)
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10. *Instructor Guide: Drug Recognition Expert Course*, supra, at 9, sect. Drug Recognition Expert 7-Day School Cannabis, session 21, p.16, 21; citing, Kari Declues, Shelli Perez, and Ariana Figueroa, A Two Year Study of  $\Delta 9$  Tetrahydrocannabinol Concentrations in Drivers; Part 2: Physiological Signs on Drug Recognition Expert (DRE) and non DRE Examinations, *Journal of Forensic Sciences*, vol.63, no.2 (2018):583-587. The article tangentially mentions green tongue through DRE results using circular reasoning for objective signs “coating on tongue” without foundation or authority.
11. Kosnoski, Edward M.; Robert L. Yolton; Karl Citek, et al The Drug Evaluation Classification Program: Using ocular and other signs to detect drug intoxication. *Journal of the American Optometric Association*, vol.69, no.4 (1998):211-227. Refers to “a greenish coating and taste buds can be raised” without reference to credible authority and empirical data.
12. Godillot, C.; A. Laprie; C. Eid, et al. Green tongue phenomenon, or diagnosis via Google, In *Annales de Dermatologie et de Venereologie*, vol.145, no.6-7, pp. 429-432 (2018). Authors substantive use of numerous qualifiers. For example, “intensive marijuana inhalation,” “exceptionally reported,” “appears to be a complication,” “sometimes advised,” “we can postulate,” “coloring green tongue may represent an indirect sign of toxic impregnation” etc.
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### Green Tongue, Probable Cause, Junk Science

## G1 Forensic Odontology—Where We’ve Been, Where We Are, and Where We Are Going: Part 2

*David Williams, DDS\*, Howard County Community College, Columbia, MD*

**Learning Overview:** At the completion of this presentation, the attendee will be able to apply the Stevens Nonprofit Lifecycles to the Odontology Section and understand the challenges and opportunities for the future.<sup>1</sup>

**Impact Statement:** This is the continuation of a study presented in a 2008 AAFS meeting, with the addition of data from 2021. It will compare the data with a “non-profit lifecycle” model as described by Stevens to help determine, within this hypothetical lifecycle, the stage of the Odontology Section and the potential challenges and opportunities for the future of the Section.<sup>1</sup>

Meeting and surpassing the challenges of a modern forensic world requires the support of a strong organization. Continued robust support for the practice of forensic odontology is critical for the science to evolve. The Odontology Section is one of the newer sections in the American Academy of Forensic Sciences, with only 2 of the 11 sections having been composed since the Odontology Section was started in 1970. Stevens proposes a lifecycle for non-profit organizations to aid officers’ and members’ understanding of the development of their organizations, which can move through stages that may include:

- **Idea:** A solution or vision for a community need has been identified and developed.
- **Startup:** The organization is out to prove the business’ case by experimenting with program design.
- **Growth:** This stage focuses on standardizing and deepening programs to meet the needs of constituents and formalizing structures and processes to ensure organizational vitality.
- **Maturity:** Formal organizational structures and processes have been established.
- **Decline:** The organization has settled into a prescribed way of doing things, slowly losing touch with clients and resisting the programmatic adaptations necessary to meet changing community needs.
- **Turnaround:** Organizations are in the process of regaining the market.
- **Terminal:** When an organization has declined too far, a turnaround is unsuccessful, or a startup never finds its place.

It is critical for members—and particularly leaders—of organizations to understand where in the cycle their organization is so that opportunities are not missed and challenges can be overcome.

This is the extension of a study presented in the 2008 AAFS meeting, with the addition of data from 2021. This presentation will provide the attendee with a comparison of the demographic data of members of the Odontology Section of the American Academy of Forensic Sciences over a 24-year period from 1997 to 2007 and also from 2022. It will additionally outline the areas in the development of forensic odontology and how these developments are related to Stevens’ Nonprofit Lifecycle theory and provide some insight into the potential future developments and challenges that may impact the discipline and what can be done to improve the likelihood of long-term survival.

A convenience sample was taken from the Odontology Section of the American Academy of Forensic Sciences Members’ directory from 1997, 2007, and 2021. The total number of section members, membership status, sex, and state/country of residence were extracted for analysis. The results will be presented so that the attendees will see the changes in the membership of the Section over the past 24 years and help identify challenges to and opportunities for the future of the Section.

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### Forensic Odontology, Membership, History



## G2 Deaths After Tooth Extraction and Treatment in the Dentist's Office in Turkey

*Huseyin Afsin, PhD, Bezmialem University, Kocamustafapasa, Istanbul, Turkey; Gulnaz Javan, PhD\*, Alabama State University, Montgomery, Alabama*

**Learning Overview:** After attending this presentation, attendees will be able to utilize the data presented in order to evaluate the prevalence of deaths after dental procedures and their corresponding criminal penalties in Turkey.

**Impact Statement:** This presentation will impact the forensic science community, the prevalence of deaths after dental procedures, and such deaths' legal consequences in Turkey.

Death is a rare occurrence in current dental practice. Despite the correct aseptic precautions taken by dentists, infection can result from various dental procedures such as tooth cleaning and filling, water contamination, and tooth extraction and root canal procedures. Such deaths are the most common emergencies that take place during dental surgery and primarily occur during tooth extraction and dento-alveolar surgery and usually occur in patients with bleeding disorders, allergic reactions, and epilepsy and because of the lack of sterilization of the medical instruments used and/or the lack of practice. Death can be caused by the obstruction of the airways because of a hypersensitivity reaction or an asthma attack, vomit, or swallowing or aspirating foreign objects such as cotton rolls or dental instruments from the operating area. Anaphylactic reactions to drugs used in dental procedures are dangerous and life-threatening pathological conditions that can lead to patient death unless the dentist is specially trained to recognize these conditions and act promptly.

This study examined 18 cases consisting of 11 men and 7 women who died because of dental treatment and whose deaths were reported by the news media. This chain of incidents started at dentists' chairs and ended in death. This study followed victims' treatments and litigation processes. Victims in 14 of the cases were under local anesthesia, 3 were under general anesthesia, and 1 was under chloroform superficial anesthesia. First interventions on 11 victims took place in private dental offices, and 7 took place in public hospitals. Dentists performed the first emergency responses on 14 of the subjects, followed by dental technicians who intervened in 3 cases, and an otolaryngologist who performed the first emergency treatment on 1 of the victims. Of the cases, 5 died because of endocarditis, 5 because of infection (sepsis), 4 because of bleeding, and 4 because of allergic reactions. In 3 cases, the victims died of a cardiovascular event following anaphylactic shock. One died as a result of nose surgery. In one case, although the otolaryngologist who performed the surgery claimed that his patient had died of poisoning from orthodontic braces, this claim was not found credible by the court, and monetary and legal penalties were levied for malpractice. Three dental technicians were given prison sentences ranging from four to five years because of the lawsuit. Dentists and anesthesia technicians who intervened during general anesthesia received monetary and legal penalties for malpractice. Other cases were found to be the result of natural causes.

As dental professionals know, the extraction of teeth can be an especially disturbing and frightening procedure; therefore, some patients prefer general anesthesia for this procedure. It is important that the dentistry practice complies and follows the minimum anesthesia standards while performing these procedures. Dental restorations performed using general or local anesthesia in elderly, disabled, or pediatric patients are not without risk, either. Outpatient dental procedures using general anesthesia are particularly risky for children because they are more likely to experience a lack of oxygen than are adults. There is also always a chance that bleeding will occur after a tooth has been removed. No deaths as the result of bleeding after normal dental surgical procedures have been reported in the literature. However, dental and medical emergency services should be familiar to staff members and available for dealing with these situations. Odontogenic and other acute oral infections can spread rapidly and dramatically. The dentist should therefore know when infections can be treated in the general practice and when the patient will be better served by a referral to a specialist or admittance to a hospital. Dentists should always comply with the routine procedure criteria and, especially, the anamnesis/indication details before and after the intervention.

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### Forensic Odontology, Death, Emergency

## G3 Dental Evidence of the Human Remains of a Slavic Soldier Population Deported to Concentration Camps During World War II

Federica Mele, MD\*, University of Bari, Bari, BA, Italy; Antonio De Donno, MD, University of Bari, Bari, BA, Italy; Sara Sablone, MD, University of Bari, Bari, BA, Italy; Mariagrazia Calvano, MD, University of Bari, Bari, BA, Italy; Mirko Leonardelli, MD, University of Bari, Bari, BA, Italy; Stefano Duma, MD, University of Bari, Bari, BA, Italy; Enrica Macorano, MD, PhD, University of Bari, Bari, BA, Italy; Valeria Santoro, PhD, University of Bari, Bari, BA, Italy; Francesco Introna, MD, University of Bari, Bari, BA, Italy

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the dental health status of soldiers during World War II.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from the dental remains of Slavic soldiers who fought in World War II.

**Aims:** Dentition is one of the most useful hard tissues in anthropological and forensic analysis because of its resistance to different environmental factors, such as postmortem modification, high temperatures, and other extreme conditions.<sup>1</sup> Teeth provide valuable information about dietary habits, general health issues, and personal identification.<sup>2</sup> The aim of this presentation was to analyze the postmortem dental records of a collection of 93 skeletal remains exhumed from the ossuary of Bari Municipal Cemetery in Apulia, Italy. These skeletal remains belonged to Slavic soldiers who were deported in 1941 during World War II to two Italian concentration camps and who died in 1946–1947. Subsequently, the bodies were buried in the Monumental Cemetery of Bari, and their human remains were exhumed in 2019 from the ossuary and inventoried in the Institute of Forensic Medicine of Bari.

**Methods:** A total number of 1,949 teeth were analyzed according to the American Board of Forensic Odontology and the World Health Organization (WHO) methods. The majority of the victims were men (95%) between the ages of 18 and 62 years. The dental evidence of these Slavic soldiers varied among different classes of age and different dental elements.

**Results:** The results showed the dental health of a population of World War II soldiers—in particular, the high rates of caries (35%), periodontal disease (61%), and dental wear (65%)—according to the high stress levels of the soldiers during the armed conflict and their subsequent deportation. It is well-known that the Holocaust dramatically affected the mental and physical status of the deported subjects and led to significant health consequences. Locomotion and cardiovascular systems were mainly affected, but very few data are reported in the literature about the dental health status of soldiers in concentration camps and of those who died during World War II.<sup>3</sup>

**Conclusions:** This is the first study reported in the literature that analyzes the effects of war and deportation on soldiers' oral health status. This analysis also confirmed the usefulness of teeth for anthropological and forensic research because of their high resistance to and preservation despite postmortem modifications and varying environmental factors.

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### World War II, Anthropological Research, Forensic Odontology

## G4 Dental Injuries During General Anesthesia: Risk Management and Forensic Perspectives

Emilio Nuzzolese, DDS, PhD\*, University of Turin, Turin, Italy; Giulio L. Rosboch, MD, University of Turin, Turin, Italy; Fabio Innocenzi, MD, University of Turin, Turin, Italy; Davide Santovito, MD, AOU Città della Salute e della Scienza, Torino, Italy; Francesco Lupariello, MD, University of Turin, Turin, Italy

**Learning Overview:** After attending this presentation, attendees will have an understanding of the risks and causes of dental damages during general anesthetic procedures and how to evaluate accidental versus non-accidental lesions due to negligence.

**Impact Statement:** This presentation will have an impact on the forensic community by presenting the retrospective analysis of dental injury claims and by offering a possible solution to reduce damage from the forensic analysis of the dental lesion and intubation dynamics via the use of a tailored and 3D-printed splint.

General anesthesia requires an endotracheal intubation performed by an anesthesiologist using a laryngoscope to maintain a patient's airway. In complex cases and in patients with dental vulnerabilities, the oral-tracheal intubation procedure can cause dental lesions. According to Givol, dental trauma and injuries represent the main causes of medicolegal compensation claims after general anesthesia.<sup>1</sup> Retrospective studies evaluate an incident rate of 0.02–0.07% of cases; according to other, prospective studies, the percentage rises to 12.1–25%.<sup>2-7</sup> The teeth most affected are the upper incisors, followed by the lower incisors.<sup>8-11</sup> The main injuries to the teeth include subluxations, avulsions, enamel injuries, crown fractures, and root fractures.

This retrospective investigation aims to quantitatively and qualitatively analyze the claims received by the Hospital *Città della Salute e della Scienza* of Turin, Italy, that were made by patients undergoing general anesthesia in the period 2011–2020, with the goal of understanding the dynamics of the traumatic events experienced and finding a possible definitive solution for patients with dental vulnerabilities.

**Materials and Methods:** In the period from 2011 to 2020, the Hospital received 576 claims for compensation, of which 47 were selected as related to dental injuries. Only 24 were found to be secondary to the oral-tracheal intubation procedure. The sample examined represents all patients treated in elective surgery, aged between 22 and 76 years, who were divided into three age categories: <35 years; 35–60 years; and >60 years. Cases were analyzed to evaluate the responsibility of the anesthetist, the oral condition of the patients, and the compensation paid. An anonymous questionnaire was also delivered to 21 anesthesiologists who were working in the same hospital to evaluate their use or misuse of the standard protective splint supplied.

**Results:** The patients were distributed according to age: Group A, with patients <35 years of age (8.4% of claims were dental related); Group B, with patients between 35 and 60 years of age (41.6% of claims were dental related); and Group C, with patients >60 years of age (50% of claims were dental related). Claims related solely to dental treatments ranged from €300 to €10100 in those cases of assessed negligence. The teeth most commonly involved were the central and upper lateral incisors (in 87.5% of the cases), the lower incisors (in 12.5% of the cases), and the lower canines (in 4.1% of the cases). The anesthesiologists interviewed confirmed either sporadic use, or even misuse, of dental protective devices in 61.9% of the cases; 66.67% of those surveyed said they would definitely use a protective splint, but that the splint should be thinner and tailored to the individual patient.

**Discussion and Conclusions:** The forensic analysis of the claims for dental damage highlights that patients' poor oral health is one of the principal causes of dental damage during intubation but that the misuse of the protective device supplied by the hospital is often a contributing factor. Once a dental vulnerability has been identified by the anesthesiologist through the collection of the patient's medical and dental history, a dental visit should be requested to evaluate the dental fitness of the patient.<sup>12</sup> Dentists should treat teeth at high risk of damage (that is, with old restorations or critical periodontal conditions) and/or proceed with a custom-made protective splint for the patient to wear prior to the endotracheal intubation, which would significantly reduce the risk of dental damage.<sup>13</sup> Per this study, the best outcome in the protection of teeth during tracheal intubation procedures can be achieved by using an intraoral scan of the patient and a custom-made, 3D-printed mouthguard.

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## **Negligence, Endotracheal Intubation, Dental Claims**

## G5 The Role of Charting Dental Anomalies in Human Identification

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**Learning Overview:** In forensic odontology, the comparative dental analysis of Antemortem (AM) dental records and Postmortem (PM) dental findings completely depends on the reliability of the dental records received. The more accurate, complete, and up-to-date the dental records are, the easier and faster the identification process will be. Unreliable dental records may lead to wrong conclusions when it comes to human identification. It is seen that only strict medicolegal and ethical codes would encourage clinicians to keep adequate dental records of patients. In numerous countries, even though laws on the maintenance of dental records exist, many dentists do not maintain adequate dental records. Through this session, the presenter hopes to create awareness among all attendees (mainly forensic professionals and clinical practitioners) on how dental anomalies can be used for human identification and how the accurate recording of every single feature/anomaly in the oral cavity can, directly and indirectly, impact human identification in the future.

**Impact Statement:** Although identification using teeth is considered to be one of the easiest, most reliable, and most economical methods of human identification, it is still not utilized up to its potential in several countries. From the survey conducted, it was observed that the awareness of forensic odontology among dentists was exceptional, but that the charting of dental anomalies was poor. This presentation (with the help of autopsy cases as examples) will prove that even a single dental feature/anomaly can help in identification if that feature/anomaly is described correctly in the form of dental charts or recorded in the form of radiographs, photographs, or casts that constitute the AM dental records. As a result of this study, “The Atlas of Dental Anomalies” and “The Scale of Forensic Significance of Dental Features” were designed to aid the charting of dental anomalies and to understand their forensic significance in human identification. This understanding would help create better, more reliable, and more up-to-date dental records that can be used for forensic identification purposes in the future and on a global scale.

An increase in the awareness of dental hygiene among people over the years consequently provoked a significant decrease in the occurrence of dental caries and, thus, a decrease in the number of dental restorations. This improvement of oral health affected the comparative dental analysis using dental treatments for human identification; hence, existing dental features or anomalies could act as unique identifying features. This study evaluated the awareness of dentists on charting dental anomalies using a dental-charting task and addressed the importance of maintaining dental records for forensic and medicolegal purposes. An online survey-based study was conducted of 101 dentists practicing in the South Indian states of Karnataka, Kerala, and Tamil Nadu using Google® Forms. Results showed that clearly visible anomalies such as midline diastema, crowding, and transposition were mentioned by only 11.8%, 22.7%, and 5.9% of the respondents, respectively. In addition, 17.8% misnamed the accessory cusp on a premolar as a Talon’s cusp. The awareness of forensic odontology among dentists was exceptional, but their dental charting needs improvement.

A “Scale of Forensic Significance of Dental Features” was created to interpret the accuracy when recording anomalies and was comprised of three parameters, namely: Incorrect answer (0%), Partially Correct answer (50%), and Accurate answer (100%). The parameters classify different levels of forensic significance of dental findings in human identification. Only a few respondents submitted an Accurate or a Partially Correct answer, and, as a result, an Atlas of Dental Anomalies ([www.theatlasofdentalanomalies.com](http://www.theatlasofdentalanomalies.com)) was created to rectify this poor pattern of dental charting, as the site can be used as study material for dental students, as a checklist for dental nurses and hygienists/assistants, and/or as reference material for dentists (or police, lawyers, or other laypeople). This site not only creates awareness of dental anomalies but also acts as a reminder of the correct terminology for those who tend to misname anomalies. The Atlas of Dental Anomalies along with the Scale of Forensic Significance of Dental Features were designed to improve the charting of dental anomalies and to explain their forensic significance in human identification, which in turn helps create better, more reliable, and more up-to-date dental records that can be used for identification purposes on a global scale.

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### Forensic Odontology, Dental Anomalies, Human Identification

## G6 Smile Selfie Images in Forensic Dental Identification: A Novel Procedure Proposal

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**Learning Overview:** After attending this presentation, attendees will understand how the identification process of human remains can benefit from the supplementary dental information detectable from smile selfie images of missing persons.

**Impact Statement:** This presentation will have a positive impact on the forensic community by presenting the results of a structured protocol on the visual comparison of images of front teeth by forensic odontologists and dentists and the protocol's application in the field of forensic dental identification.

The collection of postmortem dental evidence from unidentified human remains is fundamental to the initial reconstruction of—as well as to the definitive comparative identification of—those remains.<sup>1</sup> The main challenge of dental identification may be collecting antemortem dental data from dental clinics and hospitals. However, relevant dental data may be available from the family of the missing person by conducting a thorough interview and collecting dental information that is in the home of the missing person or victim, information that could be converted into useful secondary forensic antemortem identifiers. For example, old radiographs or dental devices or orthotics such as a bruxism bite, a sports mouthguard, bleaching trays, and orthodontic retainers are examples of these identifiers. In this regard, a portrait showing the person smiling can provide identifying features that could lead to the comparison of postmortem and antemortem dental data.<sup>2,3</sup> This study processed smile selfie images for the purpose of introducing a structured protocol as a supplementary tool in forensic dental identification.

**Materials and Method:** A sample of 253 people was recruited on a voluntary basis. Each person was asked to take a selfie while smiling and showing the front teeth. These images were considered antemortem photos found on a victim's phone or social media profiles; 71 of the 253 people were also asked to take an intraoral picture for the purpose of simulating postmortem pictures. Five odontologists were involved in a blind and random comparison of the images to evaluate the degree of recognition of the 71 images from among the 253 smile selfie images sampled.

**Results:** The visual comparison had an average recognition of 65.5%. The Shapiro-Wilk test showed normality of the study, and the Student test showed no significant differences among the observations of the operators involved. This study observed that the shift variables of the front upper incisors presented the least differences and that the axis of central and lateral incisor edges—rounded or straight—were the variables most easily identified. Conversely, the offsets of the lateral incisors in relation to their adjacent central incisors were the criteria with the most subjective differences among the operators. The features that were best recognized by all operators were the axes of the front incisors and the shapes of the central upper incisors' edges

**Discussion:** This analysis demonstrated a recognition rate of more than 50%, which is not sufficient to make this process reliable in a disaster victim identification scenario but is sufficiently reliable for application in single identification cases when also applying the exclusion criteria. The shooting angle of the photographic snapshot and the lens distortion can lead to interpretation errors when teeth do not lie on the sample plane because they are either more lingual or more vestibular. This study recognizes that visual recognition is not a reliable enough method to use in human identification, but that visual recognition can be used as a preliminary sorting tool when dental features, such as diastema, malpositions, rotations, pigmentation, crown morphology, dental piercing, and restorations, are visible on the front teeth.<sup>4</sup>

**Conclusion:** A selfie picture search on social media should be performed by forensic odontologists as adjunct data in the preliminary reconstructive identification process. Smile selfie pictures can be used in the preliminary search of compatible reported-missing persons as supplementary data and when dental records are not yet available. The proposed protocol and the current state of research on the smiling selfie are showing promising developments when combined with artificial intelligence and facial recognition software.

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### Selfie Images, Dental Identification, Dental Autopsy

## G7 Computer-Aided Dental Identification Using Intra-Oral Scanning Techniques

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**Learning Overview:** Attendees will learn the different steps of an innovative protocol for dental surface comparison, with perspectives in forensic identification. The first results of its feasibility study will be exposed.

**Impact Statement:** This presentation proposes an innovative protocol for individual identification based on comparing dental surfaces obtained by the Intraoral Scanning Technique (IOST), which represents a complementary tool in difficult Disaster Victim Identification (DVI) situations that have limited access to radio-imaging. This method would give experts quantitative decision criteria in little time and with little equipment necessary.

Because of its recent development in dental practice for prosthetic and orthodontic purposes—but also as a medicolegal tool—the IOST represents a growing and promising source of Antemortem (AM) data: It allows for the long-term preservation of dental morphology and satisfying traceability, digital storage, and transfer. For the same reasons, IOST provides promising Postmortem (PM) material that easily saves 3D-colored representations of the teeth, without contact and even in difficult conditions (burnt remains, constraint mouth opening, etc.).

In cases of airplane crashes or explosions, DVI units may face situations in which sound dental elements or fragmentary teeth are found. With limited radiological imaging available, dental identification may rely on the morphological characteristics of teeth, which is mostly a subjective approach. New methods are needed to use these criteria as objective decision tools.

Based on their acquisitions using IOST, a protocol for a computer-aided comparison of dental surfaces in an identification objective is proposed.

In a preliminary study, ten upper molars from the collection of the Forensic Science Laboratory of the French Gendarmerie (IRCGN) were scanned with a Condor<sup>®</sup> camera and then implemented in the Cloudcompare<sup>®</sup> software as .ply files. The dental mesh was converted as a pointcloud, and the occlusal surface was sectorized. Each tooth was scanned twice in order to mimic an AM and a PM sample.

The PM specimens were registered on the AM dental surfaces, and the mean point-to-point distance was calculated for match (PM and AM surfaces from the same individual) and non-match (PM and AM surfaces from different individuals) situations. Intra-operator and inter-operator variability of the method was evaluated.

First results demonstrate satisfying repeatability and reproducibility values and statistical differences between matches and non-matches (5% threshold).

Sensitivity/specificity tests are needed, using appropriate samples; and limitations because of fragmentation, attrition degree, and the use of different IOST devices have to be evaluated. Nonetheless, the proposed method shows promising perspectives, with little material or training required and in a short amount of time, making it particularly important in DVI contexts

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### Intra-Oral Scanning Technique, Dental identification, 3D

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## G8 A Catastrophic Bus Crash in Hungary—Another Disaster Victim Identification (DVI) Operation

*Ajang Armin Farid, DMD, National Bureau of Investigation, Budapest, Hungary*

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**Learning Overview:** After attending this presentation, attendees will have learned about a fatal bus crash that occurred in Hungary in August 2021, where the Hungarian International Criminal Police Organization (INTERPOL) Disaster Victim Identification Team was called in to recover, register, and identify victims.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness that well-trained DVI teams are essential for the welfare of society.

On August 15, 2021, a bus carrying 56 Hungarian tourists was on its way back to Budapest from Croatia. Two bus drivers took turns driving to and from the Croatian coast and were only an hour away from their destination in Budapest when, because of a mechanical issue, the driver lost control of the vehicle, which drifted off the highway, crashed into the pillar of a bridge, and landed on its side in a ditch. Chaos was reported by witnesses before rescue operation teams arrived at the scene and began to revive and stabilize the severely injured passengers. Eight people lost their lives, among them the driver of the bus, who was decapitated. Two others suffered severe injuries, and 46 had minor injuries. Within two hours of the accident, the DVI Interpol Hungary Team was deployed and arrived at the scene to identify and register the victims. Strict INTERPOL protocol was followed and resulted in the identification of all the victims via dental recognition.

Currently in Hungary, plans are underway to expand the contingent of forensic odontologists and to fully train them in DVI INTERPOL protocol. Because of the pandemic, there were no opportunities to meet new recruits in person and/or to establish in-person training courses for them. However, for more than a year now, regular virtual training workshops have been organized and facilitated by the Hungarian DVI Dental Unit's leadership, who have invited specialists from around the world, mostly American Academy of Forensic Sciences (AAFS) members, who have shared with the participants their learning and experiences about various DVI missions. These courses are now being offered to attendees from all continents and continue to be great sources of learning and development.

These training workshops not only offer the highest standards of education in this important field of forensic science—they also help promote a common vision among those working in the field of DVI internationally, especially in Europe.

In overcoming the challenges we face together as one human family, the concepts of accompaniment and assistance are vital in developing our capacities when chartering our own individual path for progress and growth. Great accomplishments are possible in the field of public service through unity of vision and action and ongoing mutual understanding, collaboration, and support.

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### DVI, Identification, INTERPOL



## G9 Dental Identification in a Case of Purposeful Commingling of Remains by a Toronto Serial Killer Operating in the Gay Community of Toronto, 2010–2017: The Bruce McArthur Case

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the planning and management required in the death investigation of a highly politicized case involving multiple victims of homicide attributed to a single perpetrator.

**Impact Statement:** This presentation will impact the forensic science community by emphasizing the importance of interdisciplinary and interagency collaboration in complex death investigations as well as the need to recognize and address the institutional biases inherent in these investigations that further victimize the marginalized communities being served.

Between 2010 and 2017, several men with ties to Toronto’s LGBTQ+ neighborhood known as “The Village” went missing. Two consecutive taskforces were created to investigate these disappearances. Ultimately, Bruce McArthur, a 66-year-old self-employed landscaper, was arrested on January 18, 2018. Police, who had been surveying McArthur, intervened when a potential victim entered his apartment and was found handcuffed to a bed. A search warrant provided access to McArthur’s electronic devices, which provided macabre details of the crimes and their aftermath. A total of eight first-degree murder charges were brought following what would become the largest criminal investigation by the Toronto Police Service and support agencies.<sup>1,2</sup>

Thirty properties previously visited or serviced by McArthur were systematically searched by police and forensic experts during a Canadian winter. Cadaver dogs focused on very large planter boxes at a residence where McArthur stored some landscaping equipment. The planters were brought to the Forensic Services Complex, where several human remains, in varying states of decomposition, were discovered concealed among the plants and soil. An exhaustive search adjacent to the properties revealed further remains.

A multidisciplinary approach, which involved the expertise of forensic anthropologists, pathologists, and odontologists, was required to analyze and identify the bodies. From an anthropology standpoint, the case was complicated because all the victims were of the same sex and because the remains had apparently been purposefully commingled, with some body parts, including two skulls, missing. Eight deceased victims were subsequently identified using a combination of fingerprint, DNA, dental, and skeletal comparisons, often with several means of identification used to identify each individual. Antemortem dental records were obtained for seven of the victims.

Toronto’s LGBTQ+ community has had a long-standing history of distrust of the Toronto Police Service. The Bruce McArthur investigation has spurred an external review of how police have handled missing-person cases. Advocates within the community are hopeful that the review will identify the systemic biases that have negatively impacted vulnerable populations, including racial, LGBTQ+, and recent immigrant groups, populations of which McArthur’s victims were members.<sup>3</sup>

The expansive investigation has reinforced the need for interagency cooperation and the need to ensure that adequate numbers of trained individuals can work together in a careful, expeditious manner in marginalized communities.

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### Forensic Odontology, Identification, Serial Killer

## G10 The Digitalization of Dental Arcades in Forensic Dentistry

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**Learning Overview:** This work aims to demonstrate the feasibility of using 3D digitization of dental arches as a subsidy for the anthropological examination of human remains.

**Impact Statement:** The total time to digitize and create each 3D model in dicom format is about five minutes per arc; once the image is collected, it can be compared immediately or sent over the Web from anywhere in the world for analysis and comparison without the need to mobilize human or bone parts.

**Introduction:** Forensic dentistry deals with the examination and evaluation of the characteristics of intact or fragmented dental arches, among other characteristics. Forensic dentistry is essential for identifying living or deceased individuals with different means of death, which is essential in mass disasters. This work scanned the dental arches of cadavers to compare the forensic dentist's report obtained by the typical physical method and the 3D-imaging method using a 3D scanner.

**Methods:** To create a 3D image of the dental arch, a 3Shape TRIOS® scanner was used. The total time to digitize and create each 3D model in dicom format was about ten minutes (five minutes per dental arch). An Instituto Médico Legal de São Paulo (IML/SP) dental examiner and specialist was asked to prepare a legal dental report, and the five dicom models were analyzed by the respective professional using a 3shape 3DViewer.

**Results:** According to the evaluation of the forensic dentist, by comparing the legal dental report carried out in the five cases to the corresponding 3D-digitized images, it was possible, via image analysis, to determine individual characteristics that could help in the identification process.

**Conclusion:** The 3D images helped in cases of unknown and/or unclaimed bodies because it was possible to carry out the reconstruction from the 3D digital scanning and send the images obtained to specialized centers for analysis, without needing to forward the remains, thereby substantially minimizing the costs of transport, time, packaging, chain of custody, and preservation of human parts. After the images are sent over the internet, even with a smart phone, they can be analyzed in specialized centers anywhere in the world.

### Images Taken after Digital Scan



### 3D Scanning, Forensic Identification, Intraoral Scanners

## G11 Second Expert Opinion in Human Identification Through a Virtual, Digital, and Remote Dental Autopsy

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**Learning Overview:** After attending this presentation, attendees will understand the advantages of teleconsultation in forensic odontology when a second opinion on a dental autopsy could be helpful or appropriate.

**Impact Statement:** This presentation will impact the forensic community by presenting the innovative process of a virtual, digital, and remote dental autopsy.

The principle behind the human identification process is comparing antemortem to postmortem evidence and the findings from the primary identifiers: fingerprint, DNA, and dental data. The collection, analysis, and interpretation of dental information require experienced dentists with a forensic odontology background and knowledge of disaster victim identification. Although forensic odontology is taught in most countries, forensic odontologists are not always available or recruited systematically for the human identification process. Furthermore, when the nationality of the deceased is unknown or in very challenging cases, a second expert opinion could be decisive in the correct interpretation of dental treatments found in the mouth of the deceased.

The process of virdentopsy™ proposed by the Human Identification Laboratory (HIDentiLab) of the University of Turin, Italy, was employed to verify the feasibility and efficacy of transmitting postmortem dental data from human remains in order to obtain a second expert opinion from forensic odontologists situated in Italy<sup>1</sup>. “Virdentopsy™” differs from “virtopsy” because the former is based on a different process and workflow, which also includes live streaming.<sup>2</sup>

Forensic odontologists from India, Peru, Syria, Sudan, and Pakistan performed a traditional dental autopsy, then transmitted the following postmortem dental data to HIDentiLab: photographs, video recordings, and X-ray images of dental arches and skulls.<sup>2</sup> The data received were analyzed, and a report was elaborated using the biological dental profile of the human remains, inclusive of dental age estimation.

The remote evaluation performed by experts in forensic odontology and disaster victim identification can be a concrete contribution to achieving timely and effective personal identification in those scenarios in which dental identifiers have been collected.<sup>3-6</sup> Examples are the victims of migrations, the unidentified human remains of unknown nationality, and when second expert opinions are needed.<sup>6</sup>

Virtual, digital, and remote dental autopsy broadens the horizon of the forensic casework involving odontologists from various countries without the need for them to be onsite and could become a standard practice for each unidentified dead body recovered or for age estimation. This procedure is a possible solution when forensic odontologists are not available at the mass disaster site, when recovery is needed, or when a second expert opinion is needed to allow for the widening or confirming of dental findings, such as age, habits, and dental treatments, thus reducing bias. This process can be a useful tool in archeology and paleopathology, for training in forensic odontology courses, or when using live streaming smart glasses and augmented reality. Per this work, virdentopsy™ should also be considered a humanitarian forensic odontology tool that can support countries in need of teleconsultations in forensic odontology, given the extremely low cost of the procedure.

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### Virdentopsy, Dental Autopsy, Human Identification

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## G12 Identification Via a Digital 3D-Simulated Dental Cast: A Case Report

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**Learning Overview:** Attendees of this presentation will learn how to seek the existence of digital casts from the antemortem dental record, obtain those files, manage the dynamic 3D image file, and compare the files to postmortem findings.

**Impact Statement:** The forensic community will be impacted by this presentation because it will increase awareness of the prevalence of this relatively new dental technology and will teach how to repurpose this technology for forensic dental identification.

The use of digital impression scanners is becoming more prevalent in modern dental practice to replace physical study models of dentition, to fabricate both fixed restorations and removable prostheses, and to perform orthodontics. Traditional plaster casts are often destroyed in the fabrication process, discarded because of the lack of storage space, or simply lost. On the other hand, 3D dental digital files and the resulting 3D-simulated casts produced from these files are generally electronically and systematically stored and can be located and transferred readily. In this case, a thorough examination of the presumed individual's dental records elicited the existence of these files, which were then easily obtained via email from the treating dentist.

This case report details the positive identification of a presumed individual through the comparison of antemortem digital, 3D-simulated study casts to postmortem photographs. Although limited antemortem radiographs existed, the sole use of radiographic comparison for positive identification was hampered because no dental restorations existed and no removable prostheses were recovered. Using a dynamic 3D image file of the dentition, it was possible to exactly match the orientation (head rotation, pitch, and yaw) captured in the photographic evidence collected at postmortem examination. The resulting "still" images from the digital casts were then compared to the postmortem photographs via side-by-side comparisons and digital overlays, which significantly increased the number of concordant points and boosted the level of scientific certainty of a positive dental identification.

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**Identification, Digital Dental Cast, 3D Scanner**

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## G13 How Scientific Evidence Can Lead to Human Identification Without a Corpse

*Luciene Corradi, MS\*, IMLAR, Belo Horizonte, Minas Gerais, Brazil*

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**Learning Overview:** Attendees will learn how forensic dentistry/forensic anthropology assumes a particular role in the contexts of determination and clarification of facts involved in litigation, leading to its conclusion in cases of human identification.

**Impact Statement:** This case report will demonstrate how forensic human identification can effectively corroborate the opinion of the police authority in relation to the existence (or not) of facts relevant to the investigation of a crime.

In Brazil, forensic medicine is a tool for police investigations that provides technical information and support for certain case facts and clarifies their criminal character. In the same way, forensic dentistry represents, in specific situations, the only method through which to obtain the factual background of the criminal act, converging to a conclusion of the investigation in which the facts have been determined and penal legislation may be applied according to current Brazilian Criminal Law.

This case report, realized by the Serviço de Odontologia Legal do Setor de Antropologia Forense (SAF) do Instituto Medico Legal de Belo Horizonte, Estado de Minas Gerais, Brasil (Forensic Dentistry of Forensic Anthropology Department of the Legal Medical Institute of Belo Horizonte, Minas Gerais/Brazil), will demonstrate how forensic human identification can effectively corroborate the forming of the “expert opinion” of the police authority in relation to the existence (or not) of facts relevant to the investigation and how it can play a specific role in the context of determination and clarification of the facts involved in litigation, leading to its conclusion.

**Case Study:** A lower Removable Partial Denture (RPD) was sent to SAF by requisition for anthropologic dental examination. The prosthesis was allegedly found, according to the requisition information, at the locale where the homicide occurred and the corpse was hidden. These facts were determined through the work of the designated police authority and their team, who additionally found that the probable perpetrators of the crime had also placed a dental prosthesis belonging to the victim in a hidden place at the locale. As a further result of the investigation, the police were led to the discovery of a missing person report filed on the same date as the crime. Thus, the police investigation team was able to collect data and characteristics of the missing person, including dental records in the form of radiological images of some of the victim’s teeth. In this case, all of the dental documentation that was found to belong to the missing person was sent (along with the partial denture recovered at the locale of the crime) to be analyzed, with the objective of formulating an expert opinion—after genetic material (on a swab) from the prosthesis as well as from relatives of the supposed victim was collected and analyzed—as to the whether the prosthesis belonged to the supposed victim. There were two prosthetic crowns fixed on the RPD that were studied in radiological images. The comparative study of these images, along with radiological images from the teeth of the dental chart sent, showed that they were absolutely compatible. The result of this comparative dental study in human identification was finalized successfully, permitting the police investigative team to materially conclude that the person was indeed the victim of homicide, even though the body was never found.

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**Human Identification, Anthropology, Forensic Dentistry**

## G14 The Role of Dental Pathologies, Anomalies, and Restorations in Forensic Identifications: Its Applications to Aajnala Dental Remains

Jagmahender Singh Sehrawat, PhD, Panjab University, Chandigarh, India; Priyanshi Sood, MSc, Panjab University, Chandigarh, India

**Learning Overview:** After attending this presentation, attendees will realize the importance of dental anomalies and pathologies and their antemortem record in postmortem examination of the deceased.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that dental anomalies, restorations, and pathologies can be valuable adjuncts to the forensic identification of unknown human remains.

Teeth are the hardest and most resistant structures of the human body and retain their unique forensic significance for a comparatively long period of time, even in the worst conditions of taphonomic degradations resulting from biological or chemical destruction. In addition to the unique odontological, molecular, and chemical characteristics of teeth, their dental pathologies and structural anomalies play crucial roles in the forensic identification of unknown human skeletal remains. The idiosyncratic features of teeth, such as staining/coloration patterns, developmental defects, wear and attritions, restorations/implants, cultural modifications, signs of tobacco or nut chewing, occupational stigmas, etc., act as valuable adjuncts to the examination of teeth in forensic or bioarchaeological contexts. Dental pathologies and anomalies may reflect oral hygiene, dietary patterns (such as consumption of sweets, sugar, fats, and proteins), and the socioeconomic, sociocultural, and occupational statuses of an individual. This work presents a brief overview of different dental defects and their putative roles in the forensic identification of unknown human remains.<sup>1-3</sup>

Variations in shapes, colors, positions, age changes, wear patterns, and restorative work make the human dentition as individual as fingerprints. Dental pathologies can thus reveal the dietary status, population affinity, and ancient or contemporary nature of teeth found in forensic anthropological contexts. Wear pattern can reflect the type of food eaten (fibrous or non-fibrous), the occupation, and the oral activity patterns (like mal-occlusions) of an individual. Dental anomalies are developmental variations (morphology, number, and arrangement of teeth) unique to an individual that are considered crucial for identifying unknown human remains found in forensic or bioarchaeological contexts. The localized insignificant/mild anomalies of restoration, crowding, and spacing give every individual a unique dental identity that may be helpful in forensic identifications. Because of their uniqueness, stability, and low frequency of occurrence in different populations, developmental dental anomalies can assist in cases of postmortem identification.<sup>1</sup> However, dental anomalies can help in human identification only if they were properly stored as antemortem dental records in the form of dental charts, radiographs, photographs, text details, or similar forms.<sup>4</sup> A dentist's awareness of the forensic value of maintaining antemortem dental records, of state regulations, of the ethical and medicolegal responsibility of the consultant, etc., will determine the records' proper handling and preservation for future use.

The negligible percentage of dental pathologies (wear, caries, and hypoplasia) in Aajnala teeth supported their military affiliation hypothesis. The recruits of the colonial army in 1857 were expected to have sound oral health and hygiene relative to that of the contemporary civilians. Low levels of carious lesions may also imply that the individuals were young and healthy at the time of their deaths. The notched incisors and canines in the majority of studied anterior teeth may result from the fact that the slain sepoy had to break the threads of pork- or beef-greasy cartridges during their military duties and strategic operations. The continuous use of anterior teeth to manipulate threads, nuts, wires, and other instruments by tailors, carpenters, cobblers, electricians, musicians, and other professionals also made them notched.<sup>5,6</sup> Ancient teeth had comparatively lesser chances of having dental pathologies than the teeth of contemporary, modern populations; the same was corroborated by pathological examination of Aajnala dental assemblage, which again endorses their possible affiliation to soldiers killed in 1857.<sup>6,7</sup>

In this presentation, different types of dental pathologies, anomalies, and restorations and their possible roles in forensic identifications, with a general reference to Aajnala dental remains, will be discussed.

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### Forensic Odontology, Dental Anomalies and Pathologies, Aajnala Dental Remains

## G15 Odontology Forensic Identification in the Case of a Flash Fire Death

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**Learning Overview:** The aim of this presentation is to focus on the importance of forensic odontology identification in cases of unidentified cadavers.

**Impact Statement:** This presentation will impact the forensic science community (consisting of the forensic pathologist, the forensic radiologist, and the forensic odontologists) through a multidisciplinary method of identifying a cadaver, especially in cases of flash fire deaths. In fact, flash fire deaths often make cadavers unidentifiable because of the strength of the flames causing the destruction of soft tissues and organs.

Forensic dentistry, or forensic odontology, is defined as the branch of forensic pathology that analyzes dental remains (whether fragmentary or complete and including all types of dental restorations) from unknown persons or bodies, with the goal of possibly identifying the latter. Indeed, dental identification is an important postmortem identification method. In some cases, it is the only method that can be used to make or refute the identification of unrecognizable corpses because the teeth remain the only parts of the body not destroyed, such as in cases of mass disaster, incineration, or severe decomposition of the corpse.<sup>1</sup> Indeed, flash fire death is an uncommon death related to severe damage that destroys the body. The majority of victims die of the effects of fire in enclosed spaces (for example, a house, an apartment, a room, a barn, a garden shed, an auto, a mobile home, or a ship). Gerling et al. classified the damage induced by fire in three degrees: Grade A—little soft tissue loss, with rupture of the abdominal wall; Grade B—moderate soft tissue loss, especially in the lower extremities, with the opening of the chest and/or abdominal cavities but sparing the head; and Grade C—soft tissue loss in the lower and upper extremities, with the opening of the cranium, sometimes in combination with the opening of the chest and/or abdominal cavities.<sup>2</sup>

**Case Report:** In December 2020 in the south of Italy, a burning car was noticed inside a warehouse. Firefighters arrived on the scene, and after extinguishing the flames, they found body remains inside the car. The prosecuting officer consulted the coroner. On the crime scene, the car, completely destroyed, contained an unidentifiable body. External examination of the cadaver revealed a classification of C-grade wounds according to Gerling: The face was unrecognizable, with the opening of the skull, chest, and abdomen, and loss of the lower and upper extremities. At autopsy, it was discovered that the tongue, pharynx, and especially glottis were scorched. The lumen of the larynx, trachea, and main bronchi were thickened, too. In addition, there was soot in the tracheal mucus, a sign of breathing while the fire was in progress.<sup>3</sup> Histological examination of the lungs and airways in Hematoxylin and Eosin (H&E) showed elongated cylinder epithelia, elongated cell nuclei, reactive hyperemia in the subepithelial tissue, and peripheral soot particles that resulted from the heat-inhalation trauma.<sup>4</sup> A forensic dental examination was performed and showed the absences of tooth n.11 and tooth n.12 and an orthodontic appliance of teeth n.13 and n.14. Dental photos of a suggested missing man were analyzed and showed the same characteristics as those of the cadaver. The comparison of the Orthopantomogram (OPT) performed antemortem of the missing person with the postmortem of the cadaver perfectly aligned. Thus, according to the American Board of Forensic Odontology (ABFO), a positive identification (Grade 1) was achieved because of a perfect match between premortem and postmortem dental data.<sup>1</sup>

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### Odontology, Identification, Flash Fire Death

## G16 Dental Hygiene Students' Matching Accuracy When Comparing Antemortem Dental Radiographs and Oral Photos to Simulated Postmortem WinID3® Records

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**Learning Overview:** Attendees will learn about senior dental hygiene students' identification match accuracy with simulated antemortem dental radiographs and oral photographs to postmortem dental charts.

**Impact Statement:** Forensic odontology educational opportunities are not readily available in dental hygiene formal education, and little is known about the transferability of their skills to assist forensic odontologists during disaster victim identifications. Therefore, this research will fill a gap in the literature about dental hygienists' ability to assist forensic odontologists when needed.

**Objective:** Matching dental records are one of the most reliable methods for Disaster Victim Identifications (DVI). The aim of this study was to assess the ability of senior dental hygiene students to accurately match simulated cases based on Antemortem (AM) radiographs and oral photos to Postmortem (PM) WinID3® charts as a possible transferrable skill for disaster victim identification, gained during formal education. The study also compared participant accuracy when matching AM radiographs to PM dental records versus AM oral photos to PM dental records.

**Methods:** Five patient cases (labeled A–E), including a Full-Mouth Series (FMS) of radiographs and a set of five intraoral photos, were selected and de-identified from a dental hygiene care facility's patient records software system to serve as AM images. Researchers created simulated WinID3® dental charts for each case to serve as PM records for comparison. Cases varied in complexity—with cases B and E having less distinctive dental identifiers (for example, no restorations), while cases A and C had similar restorative patterns, and case D consisted of numerous restorations that varied greatly from those of the other cases. A convenience sample of  $N=31$  senior dental hygiene students from one institution were presented with a PowerPoint® explaining how to interpret WinID3® dental charts; they then were presented with the five mismatched cases and asked to visually interpret each case to make ten total identification matches: five based on AM digital FMS, with simulated PM WinID3® records, and five based on AM digital photos with PM WinID3® records. Participant match accuracy per image type was assessed for performance differences among radiographic and photographic matching abilities. A researcher-designed Qualtrics post-test asked participants to indicate perceived difficulty and confidence in making matches per image type. SPSS software was utilized for statistical analyses of this Institutional Review Board (IRB) -approved descriptive study.

**Results:** Participant match accuracy for cases with numerous dental identifiers (cases A, C, and D) ranged from  $M=93.5$  to  $M=77.4$ . Match accuracy declined with cases B and E of less distinctive dental identifiers ( $M=58.1$  to  $41.9$ ). Participants commonly mismatched case A with C, and case B with E when comparing images to charts. McNemar's chi square revealed no statistical differences in participants' match abilities depending on image type (radiographs vs. photos):  $p=.687$  (case A),  $p=.388$  (case B),  $p=.625$  (case C),  $p=1.000$  (case D), and  $p=.774$  (case E). Most participants (74.2%) indicated photo matching as more challenging than radiograph matching despite quantitative findings showing that matching performance was not statistically different depending on image type. Most participants (70.9%) indicated experiencing no to slight difficulty when attempting to match radiographs to charts, and 87% indicated slight to moderate difficulty when matching photos to charts. When asked about perceived confidence, 93.5% indicated they were moderately confident that they had correctly matched at least half of the sets.

**Conclusions:** The results of this study demonstrate that dental hygiene students can accurately match simulated AM images with PM records with confidence. While much research has been performed to validate the uniqueness of dental radiographs, oral photos, and charting for victim identification purposes, this study is innovative because of its focus on assessing the ability of dental hygiene students to accurately match AM and PM records. Forensic odontology educational opportunities are not readily available in dental hygiene formal education, and little is known about the transferability of these skills to assist forensic odontologists during DVI. More research is needed in education and practice when preparing dental hygienists for forensic-based service.

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### Forensic Odontology, Dental Hygienist, Identification Match Accuracy



## G17 Histological and Elemental Markers in Teeth Help Reconstruct Decedent-Specific Lived Experiences

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**Learning Overview:** Attendees are expected to learn about a novel and easy-to-implement method that could be used to identify, from dental remains, specific life events that may be unique to a decedent.

**Impact Statement:** Rather than relying on diagnosing visible stress lesions in unidentified skeletal remains, the methods presented here would work toward identifying more specific lifestyle factors that may be unique to a decedent's experience and help determine the decedent's identity. By recognizing signatures of stress at a higher resolution than macroscopic trauma, non-skeletal pathologies (such as systemic illnesses), and reproductive history, this method could help in restoring unidentified victims to their families and loved ones. This research improves the forensic anthropology identification process by presenting an additional method for discerning antemortem information that can not only help establish a positive identification but also limit potential incorrect matches.

This project reports on the utilization of histological and elemental markers contained in dental microstructure to infer decedent-specific lived experiences (trauma, illness, and reproduction). Thousands of long-term unidentified individuals are listed in the National Missing and Unidentified Persons System (NamUs), resulting in what has been referred to as the Nation's "silent mass disaster."<sup>1,2</sup> A significant portion (56.28%) of the active NamUs unidentified persons cases are comprised of partial (36.6%) or complete (19.68%) skeletal remains.<sup>2</sup> To address the problem of identifying unidentified individuals, this work tests the hypothesis that dental histology of cementum can be used as a record of an individual's medical and reproductive history. From histological examination of dental remains, this study proposed, for instance, to estimate the number of live births a female has had, the age of her menopause, and the age at which a male reached sexual maturity. This study performed histomorphological analyses of 47 teeth from 15 individuals with known life history events and was able to detect reproductive events and menopause in all females.<sup>3</sup> This study also found that stressful events such as systemic illnesses and incarceration were detectable. Using a novel analytical method, this study was able to time all such events with high accuracy (R-squared=0.92). Nonetheless, these promising results are limited because they do not appear to be event-specific: This study could not determine the cause of the stressor from histological analysis alone. This study, therefore, proceeded to measure, via field-emission scanning electron microscopy and energy-dispersive X-ray analysis, major and minor element concentrations across the cementum transects of 34 teeth of a model organism (Rhesus macaques) with the aim of discovering event-specific elemental signatures. The results of this analyses indicate that the relative concentrations of Magnesium (Mg) and Oxygen (O) distinguish ( $p < 0.05$ ) reproductive events from all other physiological states; Sodium (Na) concentrations are significantly altered ( $p < 0.05$ ) in association with skeletal trauma; and Mg, Na, Calcium (Ca), and Phosphorous (P) concentrations provide a unique elemental profile ( $p < 0.05$ ) in association with suckling and weaning. These results suggest that combined histological and elemental evidence may help guide investigators in their attempts to identify missing and unidentified persons. Specifically, these novel methods could be used to identify specific life events that may be unique to a decedent should the medicolegal and investigative system be able and willing to recover such details from next of kin or medical records.

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### Dental Cementum, Dental Histology, SEM/EDX

## G18 The Use of Computer Image Recognition and Interpretation Software (CIRIS) for Automation of Dental Age Assessment: An Update

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**Learning Overview:** After attending this presentation, attendees will understand why CIRIS can be used to address some of the concerns raised by the current methods used to assess a forensic age interval by dental means.

**Impact Statement:** This presentation is the next step in the goal of using these biometric algorithms to create a new population-based data table with a greater degree of granularity. In addition, by using the identical standardized computer algorithm to collect both the population data and the staging of a dental radiograph, any concerns about the accuracy of the staging comparison process can be eliminated.

Forensic age assessment by dental means estimates an individual's chronologic age interval through the scientific evaluation of the individual's dentition and maxillofacial structures. Although numerous methodologies are available, the most common method compares the progressive morphological, developmental changes of the crown, root, and apex of a tooth to known population tables. This assessment is performed by a highly trained forensic odontologist who must make a qualitative assessment of discernible radiographic changes because of progressive mineralization of each tooth. The odontologist must then assign "developmental stages" to each tooth to compare them to suitable population-specific reference data. Because dental age assessment requires skill, training, education, and experience to execute correctly, concerns of inter-operator and intra-operator consistency have been raised.

In 2009, Aschheim, Tannenbaum, and Nakhmani presented to the AAFS a paper on "The Use of Computer Image Recognition and Interpretation Software for Automated Dental Age Determination—A Proof of Concept."<sup>1</sup> The paper's premise was that using CIRIS could interpret visual dental development images. In addition, the software could derive meaningful metric information from the data via an image segmentation system. Improvements in the software, especially for its use in homeland security for real-time facial recognition and automated interpretation of satellite data, have led to a desire to revisit this technology and see whether these improvements could be incorporated into updated software.

Deep learning, which can extract high-level features, is an extremely popular tool in image recognition. It also can be used in teeth staging. With enough training data and a correct hyperparameter, we can use a good model to predict teeth stages. Data augmentation helps when the data number is limited. In imaging, translating, cropping, rotating, flipping, and adding noise can be used to increase the amount of data. Histogram equalization is an image-processing method that increases the global contrast and can solve the brightness problem. Alex Net is used with an augmented training set, and all of the code is written in Python<sup>®</sup> language.

This presentation will expand on the previously presented proof of concept application to determine the computer's ability to accurately discern meaningful biometric data utilizing deep learning. Furthermore, it will evaluate if the newer system can better stage teeth from a dental radiograph for comparison with relevant population-specific reference data.

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### Dental Age Assessment, Forensic Odontology, Computer Image Recognition

## **G19 Ancestral Differences in Dental Development**

*Graham Roberts, MDS\*, King's College London, Lambeth, London, United Kingdom; Victoria Lucas, PhD, King's College London, Lambeth, London, United Kingdom*

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**WITHDRAWN**

## **G20 Accuracy and Precision in Dental Age Estimation**

*Victoria Lucas, PhD\*, King's College Dental Institute, London, United Kingdom; Graham Roberts, MDS, King's College Dental Institute, London, United Kingdom*

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**WITHDRAWN**

## G21 Deep-Learning-Based Age Estimation From Panoramic Radiographs: Unraveling the Learning Process

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**Learning Overview:** After attending this presentation, attendees will understand which information is used from panoramic radiographs for age estimation by convolutional neural networks.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into how machine learning algorithms interpret panoramic radiographs for age estimation.

**Background:** Dental age has been proven to be a good predictor of chronological age, especially in children, for whom the permanent teeth are still developing.<sup>1</sup> In adolescents and young adults, the dental age is still useful as a predictor of chronological age, albeit less accurate and precise than it is in children.<sup>2</sup> Numerous dental age estimation methods have been described in the literature, both for children, on one hand, and for adolescents and young adults, on the other hand.<sup>2</sup> Traditionally, the methods are based on staging dental development, which is done by expert observers. Still, inter-observer variability remains, regardless of the observer's experience. To overcome the issue of inter-observer variability, automated dental age estimation methods were developed.<sup>3-7</sup> Vila-Blanco et al. demonstrated that their Convolutional Neural Network (CNN) for age estimation focused on different parts of the panoramic radiograph, depending on the age category of the studied individual.<sup>7</sup> This grossly corresponds with how human observers interpret the panoramic radiograph: by focusing on the developing permanent teeth in children and on the developing third molars in adolescents and young adults. At the same time, Banar and Bertels et al. showed that their CNN for third molar development staging benefited from background removal as a potential compensatory mechanism for having a limited amount of training data.<sup>8</sup> Therefore, it can be hypothesized that CNNs for age estimation can be aided by directing their focus inside the panoramic radiograph corresponding to a human observer's approach. Providing the observer with extra information about where to look may increase accuracy and precision by eliminating redundant information.

**Purpose:** To study the effect of three preprocessing steps of the panoramic radiographs on age estimation performance.

**Materials and Methods:** A set of 3,266 digital panoramic radiographs was collected retrospectively at *blinded for review*. The study population was between 1 and 24 years old, approximately evenly distributed between both sexes and among all age categories of 1 year.

Two preprocessing steps were conducted by a human observer: (1) cropping the panoramic radiographs to display only a rectangle within the third quadrant, and (2) indicating the long axes of the seven permanent teeth and the four third molars on the panoramic radiographs. Based on those long axes, a third preprocessing step was conducted using the Python OpenCV package: (3) masking all redundant information in the panoramic radiographs, keeping rectangles around the seven permanent teeth and the four third molars. Subsequently, the EfficientNet-B7 CNN pretrained on ImageNet was used in combination with an additional global average pooling layer and two dense layers to predict age.<sup>9</sup> Age was predicted based either on the original panoramic radiograph or on one of its three preprocessed variants. In each setup, the CNN was tested on the same random subset of 170 subjects. The remainder—for which the number of subjects could vary slightly between the different preprocessing steps because of technical constraints—was used for model fitting, including an internal validation set to monitor convergence.

**Results:** The mean absolute error equaled 0.95 year using the original panoramic radiograph, 1.15 years using the cropped images, 1.08 years using the images with the long axes, and 1.22 years using the masked images.

**Conclusion:** Rather unexpectedly, the CNN benefited from being able to consider the original panoramic radiograph. This suggests that significant discriminative information is present throughout the full panoramic radiograph and/or that directing the CNNs' focus using prior human experience could be harmful outside the limited data regime.

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### Deep Learning, Dental Age Estimation, Subadult

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## G22 Age Estimation Using Third Molar Maturation Based on Demirjian's Criteria

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**Learning Overview:** After attending this presentation, the attendees will have a better understanding of the use of third molar maturation in human age estimation.

**Impact Statement:** This presentation will inform the attendees and the forensic science community of the utility of third molar maturation in ascertaining whether an individual has attained the medicolegally significant ages of 16 and 18 years.

Forensic age estimation is one of the most important aspects of human identification. Age estimation is routinely done by assessing the age-related changes that occur in skeletal and dental anatomical regions. Most of the commonly employed methods of age estimation include assessing the epiphyseal fusion of bones and evaluating the extent of maturation of the human dentition. Teeth follow a predictable pattern of eruption and maturation that can be assessed radiographically in the living, thus allowing experts to estimate an individual's age. Third molars develop much later than the other permanent teeth, and this fact can be used to estimate the ages of individuals nearing the legal age of maturity. The ability of third molar maturation when graded using Demirjian's criteria to indicate whether an individual has attained the medicolegal ages of 16 and 18 years has not yet been verified.<sup>1</sup> The present study involved assessing the third molar maturation of 300 individuals (150 males and 150 females) of a training set using Demirjian's scoring system, generating regression models to estimate age using the third molar maturity scores, applying these models on a test set of 40 individuals (23 males and 17 females), and calculating the Mean Difference (MD) and the Mean Absolute Error (MAE) between the chronological and the estimated ages of the test set participants. It was observed that the lowest mean difference and the mean absolute error were observed for the maturation of the left mandibular third molar (MD=1.05 years, MAE=2.09 years). Boxplots generated in the study report that the third molar maturation stages given by Demirjian can identify whether an individual has attained the medicolegally significant ages of 16 and 18 years of age. The present study concluded that age estimated from third molars using Demirjian's criteria can be used to estimate the age of individuals nearing the legal age of majority. This method can be used on its own when other, well-established methods of age estimation cannot be applied or as an adjunct to these methods.

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### Forensic Odontology, Dental Age Estimation, Third Molar

## **G23 Magnetic Resonance Imaging for Multi-Factorial Age Estimation Using a Bayesian Approach: A Validation Study in Two Independent Samples**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the validity of Multi-Factorial Age Estimation (MFA) models based on Magnetic Resonance Imaging (MRI) in living adolescents and young adults.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence on the validity of MFA models for radiation-free age estimation in the living.

**Background:** The use of medical imaging for forensic age estimation remains a controversial subject and is often labeled as unethical and imprecise. To prevent exposure of an individual to ionizing radiation, MRI has been proposed as a suitable imaging modality to visualize developing anatomical structures.<sup>1-3</sup> Moreover, to increase the accuracy of the point prediction of age, to reduce its uncertainty, and to decrease the chance of an ethically unacceptable error, MFA is preferred.<sup>1</sup> Indeed, two research groups independently demonstrated that age estimation around the age of 18 years should combine data of the third molars, left hand/wrist, and both clavicles.<sup>3,4</sup> One of these research groups developed MFA models based on Bayes' rule combining MRI data of the third molars, left hand/wrist, and/or both clavicles but reported only on internal cross-validation.<sup>4</sup> In males, combining all three anatomical sites rendered a mean absolute error equal to 1.36 years, a mean width of the 95% prediction intervals equal to 5.49 years, 94% correctly categorized adults, and 90% correctly categorized minors. In females, the corresponding results were 1.41 years, 5.91 years, 93%, and 91%, respectively. However, external validation is essential before the approach could be used in practice.

**Purpose:** To externally validate MFA models based on MRI in two independent samples.<sup>4</sup> This study hypothesized that the original models perform equally well in the external validation samples as reported for internal cross-validation.

**Materials and Methods:** The relevant anatomical structures were studied in two samples, independent of the sample used in the De Tobel study.<sup>4</sup> Note that the validity of the models can be studied only if the MRI protocol applied in the validation sample is equal to (or at least very similar to) the MRI protocol applied in the original sample.

In a first validation sample, which will be referred to as the Ghent sample, MRI of the left wrist and both clavicles was prospectively conducted in 108 healthy Belgian Caucasian volunteers (52 males, 56 females), with ages between 16 and 21 years.<sup>5</sup> In a second validation sample, which will be referred to as the Graz sample, MRI of the third molars, left hand/wrist, and both clavicles was prospectively conducted in 337 healthy Austrian Caucasian males, with ages between 13 and 25 years. Development was staged and age estimation performance was tested, calculating mean absolute errors, 95% prediction intervals and their coverage, and percentages of correctly identified minors and adults.<sup>4, 6-8</sup>

**Results:** Because this is ongoing research, no results that confirmed or rejected the hypothesis were available at the time of this submission.

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### **Age Estimation, Magnetic Resonance Imaging, Bayesian Statistics**

## G24 Ethnic Variations in the Development of Third Molars Between Chinese and Hispanic Children and Adolescents

Jayakumar Jayaraman, PhD, MDS\*, Virginia Commonwealth University, Richmond, VA

**Learning Overview:** Following this presentation, attendees will understand the influence of ethnic variations, particularly between the Chinese and Hispanic populations, in dental development of third molars. This will be presented in a form of a research study that compares the average age at attainment of crown formation stages in the maxillary and mandibular third molars.

**Impact Statement:** This presentation will impact the forensic community by demonstrating variations observed in third molar development between two distinct ethnic populations. It also emphasizes the importance of using population-specific reference standards for accurate age estimation.

Age estimation is an important procedure in forensic practice, and several studies have shown relatively accurate estimates of age using dental development. The influence of ethnicity on dental development and age estimation has been widely debated.<sup>1,2</sup> A recent study demonstrated significant differences in dental maturation between Caucasian and Chinese populations, with the latter showing advanced development by over five months.<sup>3</sup> Although the relationship between ethnicity and dental development has been well established, such comparison has not been conducted in the Hispanic population.

This study aimed to compare the development of third molars between Chinese and Hispanic children and adolescents. Dental panoramic radiographs belonging to healthy children of Hispanic and Chinese ethnicity were obtained from the archives of teaching hospitals in Hong Kong and Texas, respectively. In total, 600 radiographs for Hispanics and 1,200 radiographs for Chinese children ages 6 to 17 were obtained, comprising an equal number of males and females in each age group. All third molars in the maxillary and mandibular arches were scored according to Demirjian's classification of tooth development stages (A to F) by a single trained and calibrated examiner (JJ). The radiographs were scored in the Microsoft® Access database designed by the Dental Age Research London Information Group (DARLInG). The average Age of Attainment (AoA) for each stage of development, along with the number and standard deviation, were calculated. Statistical significance was set at  $p < 0.05$ , and an independent sample t-test was used to compare the AoA for individual stages of development for all third molars, independently for females and males in the Chinese and Hispanic ethnic groups. For males, Hispanic children consistently demonstrated advancement in dental development by 0.81 year for maxillary and 0.91 year for mandibular third molars. For females, advancement of 1.16 years and 1.10 years were observed for maxillary and mandibular third molars, respectively, and the differences were statistically significant for all stages and for dentition ( $p < 0.05$ ).

In conclusion, significant advancement in third molar development was observed in Hispanic compared to Chinese children and adolescents. The variations in dental development should be taken into consideration during age estimation procedures, and a population-specific reference dataset is recommended in the considered population groups.

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### Dental Development, Ethnicity, Third Molar



## **G25 The Reproducibility of Third Molar Development Stage Allocation on Panoramic Radiographs and Cone Beam Computed Tomographs (CBCT): Comparing Imaging Modalities, Observer Experience, and Observer Calibration**

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**Learning Overview:** After attending this presentation, attendees will understand why CBCT is of added value to third molar development stages in adolescents and young adults and how observer variability affects the staging.

**Impact Statement:** This presentation will impact the forensic science community by providing well-founded argumentations on the preferred imaging modality and the approach to allocate third molar development stages.

**Background:** The clinical introduction of CBCT allowed obtaining high-quality tooth images in three orthogonal planes, using low radiation doses.<sup>1,2</sup> Consequently, CBCT is making inroads into medicolegal age estimation.<sup>3,4</sup> Although tooth development staging techniques were established on Panoramic radiographs (PAN), it is assumed that using them on CBCT provides comparable results. However, the scientific proof is scarce on stage allocation being exchangeable between PAN and CBCT. Franco et al. reported congruent developmental stage allocation results between PAN and CBCT in third molars.<sup>3</sup> Nonetheless, the early developmental stages were not studied, and a very small sample was used.<sup>3</sup> Similarly, Zirk et al. studied a relatively small sample and obtained inconsistent results comparing the development of the lower right third molar on PAN and CBCT.<sup>5</sup> Moreover, their conclusions were not supported by the results.<sup>5</sup>

When allocating and comparing stages within and between imaging modalities, observer experience and observer calibration play important roles.<sup>6</sup> However, to date, the reproducibility of stage allocation among research centers has not been discussed in the literature.

**Aim:** To study the reproducibility of staging of third molar development within and between imaging modalities (PAN, CBCT) among observers with different experience levels and from different international research centers.

**Materials and Methods:** In a sample of 449 healthy blinded for review subjects (221 females, 228 males), PANs and CBCTs were retrospectively selected if they were taken on the same day. The subjects were between 7 and 26 years old. Their third molars were staged according to the technique of Köhler et al., independently for PAN and CBCT.<sup>7,8</sup> At least one expert and one non-expert observer per international research center conducted the staging, with a separate stage allocation for each root. To quantify the agreements between imaging modalities and among observers, the percentages of agreement were reported, as were weighted kappas. Simple kappas quantified the agreements of whether or not a stage could be allocated to the considered root and tooth. The McNemar test was used to verify the differences in stage distributions. To detect systematic differences between imaging modalities and among observers, a Wilcoxon signed rank test was used.

**Hypotheses:** (1) High reproducibility is achieved in tooth stage allocations on root level between PAN and CBCT. (2) Observers with different levels of experience show congruent results. (3) Observers with different calibrations (i.e., from different centers) show congruent results.

**Results:** Since this is ongoing research, there were no results available to confirm or reject the hypothesis at the time this abstract was submitted.

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### **Third Molar Development, Panoramic Radiograph, Cone Beam Computed Tomography**

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## G26 Finding John “Little Zion” Doe: When the Science of Forensic Dentistry Proves Itself Above Rational Thinking

*Grace Chung, DDS\*, Clark County Office of the Coroner/Medical Examiner, Las Vegas, NV*

**Learning Overview:** Attendees will learn how a seemingly small aspect of forensics such as odontology has made significant contributions with years of scientific research—in this case, how an unidentified body can be identified not BY forensic dentistry but BECAUSE of it.

**Impact Statement:** Forensic sciences are a science because there is a method and systematic approach to its findings. Sometimes what one may think is common sense is contradicted by these sciences. It is easy and likely that the average person would follow what makes rational sense; however, we forensic scientists must trust our own research and studies to push forward what our examination of evidence is telling us. In seeking the truth, the forensic science community may not have the popular opinion, but it often will be the correct opinion. This presentation will hopefully inspire the community to continue becoming experts in their fields to have the confidence to be the non-popular contributor if need be and to show how important it is to have your voice heard.

On May 28, 2021, a body of a young male was found along Route 106 in Las Vegas, NV. Weighing just under 125 pounds and about 4'11" tall, it was evident that the decedent was a young child. The body was immediately transported to the Clark County Coroner's Office, and law enforcement was alerted and began investigations in hopes of securing an identify.

The events that followed were a whirlwind of dynamic information. Evidence was brought in, conclusions were made, then further investigation that depended on those conclusions ensued; yet an entire week went by with no identity for the boy, who now was nicknamed “Little Zion.” The chief forensic odontologist had been called upon to perform a dental autopsy and found himself becoming the leading opposer to challenge the investigators' initial findings.

One mother had seen photos of the boy and claimed him as her son. She told police that her son, along with a half-brother, had been taken by their father and that she had been unable to reach them for a few days. Immediately, chief forensic odontologist was asked to collect dental evidence of that mother's son and to compare it to the decedent's dental record. By now, investigators were already assuming that they knew the identity of the boy and began searching for the father. However, it took only a few minutes of reviewing the dental X-rays of the boy thought to be missing for him to be excluded as the decedent.

It was back to the drawing board on the search for the identify of this child. Based on his height and weight, investigators initially published a search request from the community for a Hispanic boy who was about 10–12 years old. However, the chief forensic odontologist pushed back because his dental age placed him much younger. According to calculations, he would be between 6 and 8 years of age. Although this age range seemed incompatible with the relative size of the body, the science behind dental age assessment is one the chief forensic odontologist urged investigators to follow. They changed their age search to 8–10.

It would be a full week between the time the body was found and when an identification was finally made, which was an enormous burden lifted off the shoulders of all involved with the case. Because unidentified children typically do not stay unidentified for more than 24 hours, the police were desperate, offering up to \$10,000 to anyone able to give them an identity and visiting door-to-door Hispanic families with school-aged boys. They had multiple artists attempting to create sketches of an antemortem portrait, and they finally received an updated and more accurate version from the National Center for Missing and Exploited Children. The identity of the boy was finally made on June 6. His name was Liam Husted, and he was only 7 years old.

Although the final identification was made using DNA, odontology played a major role in re-diverting the investigation when it was heading in the wrong direction. For one thing, once the chief forensic odontologist excluded the initial missing boy as the victim, investigators began searching for him and eventually found him and his half-brother both safe, camping in a remote area with their father. Also, the change of the age interval for the deceased child helped investigators look for younger children when they initially had been looking at a much older population.

Odontology is a branch of forensic sciences that can often be overlooked because of its seemingly small scope. However, as shown in this case, the science of odontology can be used as a reliable resource and can make large contributions to complex cases.

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### Age Estimation, Unidentified, Child Abuse

## G27 Bitemarks That I Have Known

*Peter Hampl, BS, DDS\*; Spokane County Medical Examiner, Spokane, WA, Pierce County Medical Examiner, Tacoma, WA, Disaster Mortuary Operational Response Team, Washington, DC, and Blake Emergency Services, Pyegreave Combs High Peak, United Kingdom*

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**Learning Overview:** It is the intention of this presentation to show the complexities of bitemark analysis and the rarity of human bitemarks of evidentiary value.

**Impact Statement:** After attending this presentation, it is hoped that a conservative approach to bitemark analysis will be forthcoming.

This presentation is a follow-up to a presentation given at the 2020 American Academy of Forensic Sciences meeting in Anaheim, CA, titled, “Non-Bitemarks I Have Known.”<sup>1</sup> This presentation covers a 40-year period of involvement in pattern injury analysis. In that period, only four cases have come to this study’s attention that were of evidentiary value. Although human bitemark evidence has been under continued criticism over the past 20 years as being unscientific, there is in rare cases very useful evidence observable to the trained odontologist. It is extremely important that the odontologist be conservative and not develop a bias during the investigation of pattern injuries.

Standard scientific methods cannot be used in pattern injury analysis. Neither the odontologist nor anyone else can control or reproduce the numerous factors involved in pattern injuries on human skin. A field of science in which controlled observations cannot be done in order to study cause and effect is called an “observational science.” For example, astrophysics is an observational science in which a person cannot change the movement or any other aspect of the sun, moon, or stars. Similarly, the odontologist must develop his/her opinions based on a sound understanding of dental anatomy, clinical experience, and keen observational skills. With these tools, the odontologist must carefully understand and correctly interpret each and every mark in the pattern injury. The use of the American Board of Forensic Odontology Bitemark Analysis and Comparison Algorithm is a most helpful tool during the odontologist’s investigation of pattern injuries.

### Reference(s):

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## Odontology, Bitemark, Observational Science

## G28 The Crash of Flight IY 626: Bitemark Identification on Victims' Bodies

Charles Georget, DDS\*, IRCGN, Pontoise, France; Ing Aime Conigliaro, MSc, IRCGN, Pontoise, France

**Learning Overview:** This presentation provides feedback on a Disaster Victim Identification (DVI) mission with specific conditions: a submarine recovery scene with two distinct identification sites and a bitemark analysis applied to the identification of implied taphonomic fauna.

**Impact Statement:** This presentation will impact the forensic science community by presenting the use of a submarine rescue vehicle for the recovery of air crash victims and the influence of Postmortem (PM) examination conditions on the DVI process. Moreover, it highlights the advantages of pluridisciplinary analysis and close collaboration between two DVI team located 800km from each other.

On June 30, 2009, flight IY 626, operated by Yemenia Airways, crashed into the Indian Ocean during a Saama (Yemen)—Moroni (Comoros) service. It hit the surface of the water while approaching its destination airport. There were 142 passengers and 11 crewmembers on board. Among them were 62 French people and 43 Comorians living in France. The sole survivor was a 13-year-old girl.

On July 4, the United States Air Force located the first fragments of the aircraft after a several-day air-sea rescue operation near Ngazidja Island. The next day, the French *Bureau d'Enquêtes et d'Analyses* (BEA) detected the signals from both flight recorders. The wreckage was lying on the ocean floor at a depth of 1,200 meters. The French Ministry of Defense decided to chart this part of the very steep ocean floor at once, and the French Navy conducted this task until July 23. Then the American submarine rescue vehicle *Remora 6000* recovered the flight recorders. It was not possible to pull out of the water the first bodies and the beacons before August 21.

However, on July 8, 21 bodies and some aircraft debris were recovered near Mafia Island off the coast of the United Republic of Tanzania. It was a surprise because Tanzanian coasts were 800 kilometers away from the crash site. Autopsies could be performed as early as July 10, at Dar Es Salaam (Tanzania).

Both in Tanzania and Comoros, the autopsies revealed PM bitemarks on some of the victims' remains. Some of the bodies recovered on the seashore of Mafia Island had deep and large wounds due to squall bites and smaller and rounder bites originating from unidentified predators.

On Comoros, the videos recorded by the submarine rescue vehicle showed that the fauna were diverse near the A310 wreckage. Crabs and spider crabs were living close to *isistius* (cookiecutter) sharks. The videos allowed the Forensic Science Laboratory of the French Gendarmerie (Institut de Recherche Criminelle de la Gendarmerie Nationale [IRCGN]) to identify the species responsible for the small and round wounds.

The management of this aircraft disaster had distinctive technical, technological, and scientific features. Indeed, for the first time, French authorities resorted to the use of a submarine rescue vehicle and had to deal with two body-recovery areas.

The submarine rescue vehicle resurfaced a Digital Fly Data Recorder (DFDR), a Cockpit Voice Recorder (CVR), and the remains of 68 victims, who were lying at a depth of 1,200 meters. After that, victim identifications were performed at two different sites, located 800 kilometers from each other, under different conditions and at different periods. Eventually, the French DIV determined the origin of PM lesions found on some of the remains.

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### Identification, Bitemark, Marine Fauna

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## G29 Bitemarks: Do They Still Exist?

*Richard Fixott, DDS\*, Redmond, OR*

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**Learning Overview:** The goal of this presentation is to discuss the diagnostic criteria for suspected bite injuries, explore how to better train odontologists, and discuss how best to use bitemark evidence.

**Impact Statement:** This presentation will improve the usefulness of recognizing an injury as a human bite.

There has been much discussion concerning bitemarks and forensic odontology, to the point where some feel there is no value in recognizing or diagnosing an injury as a human bite. Concerns include the scientific basis for identifying a bitemark, disagreements among practitioners, the lack of an error rate, and, most importantly, criminal convictions based partially or solely on bitemark analysis that have been overturned.

Is there a scientific basis for bitemarks? Multiple research projects have consistently demonstrated that a bite administered with sufficient force will create an injury. Patterned injuries can be examined, and a differential or definitive diagnosis that may include or be a human bite can be made. Recognition/diagnosis of an injury as a human bite is part of the mandate to health professionals to report abuse/neglect: the diagnosis/recognition of an injury as a bite may have evidentiary value.

Diagnosis in general is based on examination of the patient, testing, and practitioner experience. A differential diagnosis of an injury as a bite can be based on the diagnostic size, shape, and pattern criteria seen. A definitive diagnosis is based on the same criteria as well as elimination of all other reasonable causes.

Disagreements among practitioners do not mean that a particular diagnosis or medical discipline is invalid. For example, dentists can interpret a radiograph as showing a cervical radiolucency as caries, artifacts, or anatomical features. Because there are disagreements, does it follow that dentists should not be qualified to detect or treat caries? Individual physiological differences will create different responses to the same treatment, test, and/or diagnosis. Different opinions or treatment options are a reflection of physiological differences, not necessarily professional competence, and make the idea of a “bench science” -type of error rate not applicable.

What should be eradicated? Definitely the inclusion of one dentition to the exclusion of all others (which is currently expressly forbidden by the current American Board of Forensic Odontology [ABFO] Standards and Guidelines, DRM Section 3, Page 9) but not the ability to observe and diagnose an injury as a bite. The goal of this presentation is to discuss diagnostic criteria for suspected bite injuries, explore how to better train odontologists, and discuss how best to use bitemark evidence.

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### Bitemark, Odontology, Diagnosis

## G30 Progress on a National Institute of Standards and Technology (NIST) Scientific Foundation Review Assessing Bitemark Analysis

Kelly Sauerwein, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Christina Reed, MS, National Institute of Standards and Technology, Gaithersburg, MD; John Butler, PhD, National Institute of Standards and Technology, Gaithersburg, MD

**Learning Overview:** This presentation reports progress on a NIST scientific foundation review examining bitemark analysis.

**Impact Statement:** The foundational review provides an assessment of the current literature related to bitemark analysis. This report will help promote a better appreciation of the limitations of bitemark analysis among the forensic science community, stakeholders, and the general public.

Bitemark analysis involves three key premises: (1) the uniqueness of human dentition, (2) the ability of bitemark characteristics to be transferred and persist on human skin, and (3) the ability to associate a bitemark to an individual. More than 200 sources (including peer-reviewed articles, books, edited volumes, conference abstracts, and discussions held during a 2019 Bitemark Thinkshop organized by the Center for Statistics and Applications in Forensic Evidence [CSAFE]) were examined as part of this assessment.<sup>1</sup>

Relying on a suspect's dentition to associate it with a bitemark pattern found on a victim assumes that the three key premises just mentioned are reliable and true. Support for uniqueness alone, however, has proven difficult. Because the ability to distinguish one human mouth from another is possible using dental records for the purpose of identifying a deceased individual, there currently exists a lack of agreement regarding the specific characteristics for establishing uniqueness when tasked to compare dentition patterns alone. Furthermore, no population frequencies have identified the degree of variation in dental features or included measures of similarity, error, or correlation.<sup>2,3</sup> Indeed, multiple bites from a single dentition have shown significant distortions such that no two bitemarks from the same mouth appeared the same.<sup>4</sup> If bitemarks with the same dentition are not reproducible, then doubt about the possibility of unique, individual dental patterns increases.

In addition, this distortion problem hinders the ability to accurately transfer individual characteristics of the biter's dentition to the substrate.<sup>4,6</sup> Not only might two bites from the same mouth leave different marks, but any one bite may not reflect the characteristics of the biter because of distortions that can result from bite force, surface area and alignment of the dentition, tooth sharpness, elasticity of the victim's skin, movement during the biting event, and the body's injury response. All of these may prevent an analyst from reliably including or excluding a potential suspect.<sup>4,7-8</sup> Therefore, a bitemark impression in the skin would not include sufficient detail to make an identification on an individual level. Additionally, current imaging methods may be insufficient because they do not capture all the characteristics necessary for bitemark analysis; for example, these methods cannot determine the force of the bite, the bruising depth, or the movement during the bite.<sup>1</sup> Even under highly controlled experimental conditions, bitemark analysis has shown high levels of variability and incorrect identifications. Bitemarks in cases in which circumstances cannot be controlled are prone to error and unlikely to produce accurate results.

**Conclusion:** For the past 60 years, repeated calls have been made for fundamental research in bitemark analysis. From this study's analysis, there appears to be a lack of empirical data to support the premise that bitemarks are unique to individuals, that those distinguishing characteristics can be reliably transferred to skin, and that modern methods can compensate for distortions and provide accurate information to exclude or not exclude an individual as the source of a bitemark.

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### Odontology, Bitemark, Dentition

## G31 Stress on Forensic Odontologists

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**Learning Overview:** This presentation will demonstrate the stress forensic odontologists are faced with and the importance of absolute accuracy in their work.

**Impact Statement:** This presentation will provide enlightenment regarding the hard work of forensic odontologists.

The forensic dentist is no longer interested in treatments and the cosmetic aspect as much as he has engaged in difficult tasks, some of which are stressful to both physical and psychological health. Work pressure on the one hand and accuracy of work on the other hand and long hours sometimes all change the psychology of the forensic dentist ..

One of the most complex tasks of the forensic dentist is the analysis and study of bitemarks. At a time when this specialty is new in Middle East countries and specialists have begun to highlight its importance to judicial authorities and the police regarding the need to call the forensic dentist in the event of suspicion of bitemarks, an opposite trend began in the United States about the failure of this evidence to be proven, in some cases , as irrefutable evidence in the course of investigations. This would increase pressure on the forensic dentist to not make a mistake when dealing with the perpetrators on the one hand and with the living and dead victims on the other hand.

In some cases, the judge may request evidence that is not completely scientifically supported; this also increases the burden on the specialist to explain that the evidence follows several different circumstances However, the specialist is obliged to respond to the judge's request, since that request is one of his duties.

Psychological pressure may not only be related to the analysis of bitemarks; other tasks are added to it, such as estimating age. What is interesting is that pressures sometimes arise from the environment surrounding the case, if the specialist has some kind of threat to his life or career if his final report is not suitable for their interests.

This presentation will mention some examples that show how risky this profession is and how it requires absolute accuracy in the work, and how it may lead some to thinking about early retirement if matters become more threatening to the safety of this specialist.

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### **Stress, Forensic Odontologist, Hard Work**

## G32 Hot Off the Press—New Standards and Technical Reports in Forensic Odontology

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**Learning Overview:** This presentation will cover the essential American National Standards Institute (ANSI) requirements for developing standards. In addition, it will illustrate how the Academy Standard Board (ASB) and the American Dental Association (ADA) Standards Committee on Dental Informatics implement those requirements to create forensic odontology standards. It will conclude with a detailed look at the development of ADA TR1077 and ASB BPR 108.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how a partnership between forensic science and a Standards Development Organization (SDO) can create consensus-based standards and best-practices documents. These documents aim to delineate policies, procedures, and guidelines that provide a framework for ensuring that the forensic sciences deliver high-quality, reliable, and consistent results.

In 2005, Congress passed the Science, State, Justice, Commerce, and Related Agencies Appropriations Act to “conduct a study of forensic science.” Its goal was to disseminate guidelines that will ensure quality and consistency in using forensic technologies and techniques. The result was the recommendation in 2009 by the National Academy of Sciences to create a “new, strong, and independent entity” outside of the jurisdiction of the Department of Justice (DoJ) to define standards and guidelines for the forensic sciences.

Standards are consensus-based specifications, rules, guidelines, or definitions created using a well-defined review and adjudication process. Historically, Scientific Working Groups (SWGs) were created to develop these documents. One example was the creation in 2011 of the Scientific Working Group on Disaster Victim Identification, which included various forensic disciplines associated with victim identification. However, one of the shortcomings of SWGs was that they follow the formal standards development process.

In 2013, the DoJ created The Organization of Scientific Area Committees (OSAC) to assist in developing these scientifically based documents. OSAC subcommittees were tasked with taking over the work of many of the SWGs, which were subsequently sunsetted. Once these documents were created, SDOs would complete the process by publishing documents that went through vigorous consensus-based rules. In addition, OSAC created a formal registry of independently reviewed and approved SDO-published documents for recommended use by forensic specialties. Both the Forensic Odontology (FO) subcommittee and the Disaster Victim (DVI) subcommittee of OSAC were tasked with handling documents affecting forensic odontology. Since then, numerous documents such as ANSI/ADA 1058–2010 (R2015)—Forensic Dental Data Set and ADA Technical Report No. 1088 Human Identification by Comparative Dental Analysis—have been placed in the OSAC registry.

In 2014, the FO subcommittee formalized a best-practice document to assess a chronological age interval by dental means. The draft document was submitted to American Dental Associations’ Standards Committee on Dental Informatics (ADA SCDI), an ANSI-accredited SDO. ADA SCDI Subcommittee 14—Forensic Odontology Informatics’ Working Group 14.3: Human Age Assessment by Dental Analysis—completed the formal review process. This document was approved as ADA Technical Report No. 1077 for Human Age Assessment by Dental Analysis (ADA TR1077). Its stated goal is to describe best practices for estimating a chronological age interval of a living or deceased individual by analyzing the human dentition and associated maxillofacial structures.

Also in 2014, SWGDVI was transitioned into the OSAC DVI subcommittee to continue work on a set of documents from various disciplines such as DNA, odontology, anthropology, and others. These documents served as Best Practice Recommendations (BPR) in disaster victim identification. The completed draft documents were then submitted to the ASB, an ANSI-accredited SDO established by the American Academy of Forensic Sciences (AAFS). The ASB’s mission is to create high-quality, science-based consensus forensic standards, guidelines, best-practice recommendations, and white papers for the entire forensic community.

One of those documents would ultimately become ASB BPR 108: Forensic Odontology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority (ASB BPR 108). This document covers best-practices recommendations as they apply to the forensic odontology section of a mass-fatality incident team and relates to the morgue’s staffing, antemortem, and postmortem data collection, the forensic dental autopsy, and applied technologies. It also includes recommendations for the dental identification and reconciliation processes to ensure consistency and reliability.

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### Standards, Forensic Odontology, ADA



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**G33 A Morphometric Analysis of Cone Beam Computed Tomography (CBCT) Images of the Mastoid Process for Sex Determination in the Adolescent Population**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of dental radiology techniques in differentiating sexual dimorphism in the adolescent population.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of using dental radiography to measure a cranial feature that displays sexual dimorphism at critical ages.

One of the key features in assessing the biologic profile of an individual is sex. The pelvis and the skull display the greatest levels of sexual dimorphism, the pelvic bone being the most reliable.<sup>1,2</sup> Following the pelvis, the mastoid process of the temporal bone is the second-most-reliable osteological feature to determine sexual dimorphism.<sup>3</sup> The size of the mastoid process is known to show marked sexual dimorphism in adulthood.<sup>4,5</sup> Currently, it is known that there is no difference in size of the mastoid process of males and females at the pre-pubertal age that allows for sexual dimorphism of skeletal remains. This study aims to assess the age at which sexual dimorphism can be differentiated in adolescents by analyzing the dimensions of the mastoid process from pre-existing CBCT images.

Overall, males present with larger dimensions, more robust cranial and facial features, and greater muscularity. However, female skulls tend to demonstrate gracile features. After the onset of puberty, the difference between males and females becomes clearer, with males developing adult features and females retaining pedomorphic features.<sup>6</sup> On average, girls begin puberty at ages 10–11 and complete puberty at ages 15–17; boys generally begin puberty at ages 11–12 and complete puberty at ages 16–17.<sup>7</sup>

This study consists of 60 individuals between 10 and 16 years of age. The study sample was derived from the database of the Department of Oral and Maxillofacial Radiology, University of California, Los Angeles. Pre-existing CBCT images taken for clinical purposes were analyzed. Size analysis of the mastoid process performed by two observers was based on linear measurements performed between selected points located on the mastoid process that objectively yielded the largest dimension. Linear measurements were recorded for both right and left mastoid processes and included mastoid height, width, and length.

All analyses were performed using R statistical software (R Core Team 2018) and SPSS version 26. No statistically significant effect was found among age, gender, and mastoid width and length. However, a statistically significant effect of gender was found on age and mastoid height (right height p value=0.017; left height p value=0.047). The intraclass correlation coefficient between the observers for right and left height was 0.96 and 0.97, respectively. In males, the greater the age, the larger the mastoid height beginning during puberty ( $\approx$  age 13) from graphic extrapolation. Therefore, from these findings, it may be concluded that the mastoid process may demonstrate dimorphic gender features from the approximate age of 13.

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**Mastoid Process, Age Assessment, Sexual Dimorphism**

## G34 Forensic Anthropological Evaluation of Mandibular Remains and Included Furnishings

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**Learning Overview:** With this study, attendees will be able to learn about the forensic anthropological evaluation of human remains that is aimed at personal identification, the tests and techniques conventionally used in this evaluation, and the means and methods used to construct a “biological profile” that identifies the species, race, age (*perimortem*), sex, probable historical and chronological periods, possible life habits, and the social conditions of the individual.

**Impact Statement:** The impact to the science community of this presentation will be a deepening understanding of the techniques used in the forensic anthropological evaluation of human remains that is aimed at personal identification.

Individual identification represents a central focus of the forensic anthropological analysis of human skeletal remains. Advancements made in recent research include new anatomical areas of interest, technologies, mathematical approaches, and biochemical analysis and techniques, namely, modern-day isotopic investigations on human remains. The use of multi-isotope profiles (e.g., C, N, O, H, S, Sr, and Pb) from multiple body tissues (e.g., teeth, bone) helps predict the likely region of origin of unidentified human remains and provides additional items of evidence for human identification, including a decedent’s possible region of birth, long-term adult residence, history, and dietary choices. Also, the study of funerary furnishings provides details that help contextualize the historical period in which the individual lived.

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### Individual identification, Biological Profile, Stable Isotope Analysis

## G35 Let's Only Use What Really Counts! A New Formula for Age-at-Death Assessment Using Root Translucency

Joe Adserias-Garriga, DDS, PhD\*, Mercyhurst University, Erie, PA; Anthony Lanfranchi, MS, Mercyhurst University, Erie, PA; Kristine Kortnick, MS, Mercyhurst University, Erie, PA; Douglas H. Ubelaker, PhD, Smithsonian Institution, Washington, DC; Sara C. Zapico, MS, PhD, New Jersey Institute of Technology, Washington, DC

**Learning Overview:** Attendees at this presentation will acquire a thorough understanding of how to apply root translucency measurement to age-at-death assessment in order to obtain more accurate results than is possible when this parameter is used in conjunction with other postformation changes.

**Impact Statement:** This presentation will affect the forensic science community by providing a new formula for dental age assessment using root translucency.

A main contribution of forensic odontologists and forensic anthropologists to forensic investigations is their ability to assess a person's biological profile. A significant component of the biological profile is the age-at-death assessment. Age assessment can assist in narrowing the search possibilities for cases involving missing persons and unidentified decedents. The study of dental tissues has long been considered a very useful tool for age assessment; therefore, several age assessment methods involving dentition have been developed and are widely used. Dental age assessment methods in infants, children, and young adults rely on developmental changes of the dentition, whereas adult dental age assessment methods rely on postformation changes.

Lamendin et al. developed an adult age estimation method based on root transparency and periodontal recession.<sup>1</sup> Their regression formula was successfully applied to the age assessment of individuals between 40 and 70 years old. Later, Prince and Ubelaker evaluated the effects of ancestry and sex and incorporated root height into the equation, thereby developing four new regression formulas for males and females of African ancestry and for males and females of Caucasian ancestry.<sup>2</sup> In 2018, Adserias et al. adjusted these techniques by demonstrating that 1600 lux was the best light setting for analyzing root translucency.<sup>3</sup>

However, a frequent concern of researchers and practitioners was the subjectivity in the periodontal recession measurement when applying the methods established by Lamendin et al. and by Prince and Ubelaker.

The aim of this present study is to create a new formula that takes into account just one postformation change: the root translucency, which has been demonstrated to show a good correlation with chronological age. So, periodontal recession (which is considered in the formulas generated by Lamendin et al. and by Prince and Ubelaker) would be taken out of the equation.

Ninety single-rooted teeth from individuals of known age and sex were used for this study. Two observers measured root height, periodontal recession, and root translucency in millimeters. Root translucency was measured applying the 1600 lux. Intra- and inter-observer errors were recorded.

Lamendin and Prince and Ubelaker's formulas were applied to obtain an age estimate. A new formula considering only root translucency was generated, without lowering the accuracy.

Thus, the findings from this research demonstrate that even with only one parameter, it is possible to determine age from uniradicular teeth with similar accuracies (and without using previously established formulas), thereby reducing the subjectivity of the estimates.

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### Age Assessment, Postformation Changes, Root Translucency

## H1 A Triple “Suicide Pact”: A Case Report With a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will better understand the role of a thorough analysis of the crime scene, together with an accurate autopsy and appropriate toxicological test in a singular case of a triple suicide consumed within a family nucleus.

**Impact Statement:** This presentation will influence the forensic science community by providing an interpretation of the relevance of autopsy and crime scene analysis as well as the importance of additional circumstantial information to distinguish between unusual suicide and homicide impacts.

Three bodies belonging to same family were discovered in their apartment. The mother, a 77-year-old woman, was discovered lying on her bed, in her pajamas. The body was cachectic, partially mummified, composed, in the absence of external lesions or blood stains on the soma and nearby. There were no signs of defense or constriction. The clothes were unaltered. No dirt was detected in the environment. At the autopsy, through toxicological tests, the cause of death was attributed to a lethal intake of morphine in an elderly person with terminal cancer. The two other bodies (49-year-old male and 46-year-old female) were found in their bed, with blood stains and the same stab wound in the left lateral cervical region, which cut off the anterior head of the left sternocleidomastoid and damaged the vascular wall of the external jugular vein. On the clothing, there were no signs of discontinuity. The cause of death was therefore determined as acute posthemorrhagic anemia due to venous bleeding, which contributed to the assumption of psychotropic drugs and alcohol, as confirmed by the toxicological data.

In such a scenario, what are the useful elements to distinguish between homicidal and suicidal hypotheses? Numerous scenarios are possible whenever two (or more) individuals are found unexpectedly dead at the same location. The considered hypothesis could be accidents and natural diseases, suicides, or homicides. Suicide pacts usually involve only two victims and are rare, accounting for only 0.6-4% of cases of suicide overall. Joint suicides have often been provoked by serious illness in one or both of the victims, so a review of their recent medical histories may be clarifying. Thorough scene investigation, autopsy findings, as well as toxicological analysis are essential in discriminating between homicide and suicide. From the crime scene, as much information as possible should be obtained on the victim's previous character, including previous psychiatric history, in order to elucidate his or her manner of death. External examination of the body is crucial to discover signs of fighting with defense injuries or lesions on the clothes. In this case, the lack of traumatic injury on mother's body and clothing, as well as the finding of morphine, led to deeming a suicidal purpose that is supported also by the circumstantial elements such as: a recent voluntary discharge from the hospital with the presence of the hospital identification bracelet, end stage neoplastic disease with baleful prognosis, and alcohol intake. The two adult children's bodies, found together in the bed, presented a single-cut wound in the neck without any defense injuries and both were surrounded by abounding blood stains.

The anamnestic-circumstantial elements, suggestive of isolation of the family unit with little, if any, contact with the outside world, the morbidity of the relationship of the sons toward the mother, and the economic difficulties, together with the serious health problems of the mother, account for an overall dynamics of “suicide pacts” most likely motivated by a condition perceived as unsustainable and devoid of possible solutions by the small family unit.

### Suicide Pact, Homicide/Suicide, Crime Scene

## H2 A Reconstruction of Events, Including a Self-Inflicted Gunshot Wound, Leading to a Fatal Motor Vehicle Collision

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**Learning Overview:** After attending this presentation, attendees will better understand how to reconstruct the probable course of events in a motor vehicle crash in which the acutely intoxicated driver sustained a gunshot wound.

**Impact Statement:** Separation of injuries from gunshot wounds from those sustained in a terminal motor vehicle collision assists in clarifying the cause and manner of death; forensic evaluation of the ballistic and blunt force injuries can allow reconstruction of the events during the interval between gunshot wound injury and death.

A 27-year-old man, with a history of heavy drinking and recent exacerbation of chronic back pain, drove away from an attempted police traffic stop. A low-speed chase ensued, with the vehicle passing through several residential yards before colliding with a tree. The airbags deployed; the unrestrained victim was found dead. Police and emergency medical technicians saw fragmented brain on the dashboard, inconsistent with the mechanism of the crash.

Police identified two rounds, one deformed, on the floorboards beside the driver's seat. A black firearm, pointing upward with hammer cocked back, was found on the floor between the console and the passenger seat. There were unfired rounds in cup holders, and empty liquor bottles throughout the vehicle.

Multiple findings at autopsy were consistent with a vehicle crash. There was significant blunt force trauma to the head (facial fractures), chest, abdomen, and extremities. The lungs exhibited lacerations due to rib fractures and contusions. There was partial transection of the descending thoracic aorta past the subclavian takeoff, associated with dissecting posterior mediastinal hemorrhage, but no hemothorax. The liver had multiple lacerations. There were omental tears and a tear in the gastrocolic ligament; hemorrhage surrounded the transverse colon. A duodenal bruise was associated with dissecting hemorrhage between the duodenum and the pancreas and foci of retroperitoneal hemorrhage. The left femur was fractured.

In addition to blunt force trauma, the decedent had a hard contact perforating gunshot wound of the right temple, with exit above the left ear. This correlated to a fatal brain injury. Extensive skull fractures were due to both gunshot wound injury and impact; calvarial and basilar skull fractures were from the gunshot wound; facial fractures that shifted the midline of the face to the left were from blunt trauma. There was also a patterned injury, a C-shaped mark, on the right side of the neck, suggestive of an incomplete circumferential muzzle imprint without a bullet defect.

Postmortem toxicology identified markedly elevated blood and vitreous ethanol,  $0.310 \pm 0.017\%$  by weight by volume in blood, and  $0.340 \pm 0.019\%$  in vitreous humor. Blood also exhibited marijuana metabolites; vitreous humor showed a trace of isopropanol. No other drugs or drug classes were identified.

Scene and autopsy findings allowed reconstruction of the probable chain of events. The first event was probably the attempted hard contact gunshot wound of the neck, resulting in misfire. It is likely that this occurred while the victim was driving away from the attempted traffic stop; the unfired round found on the floorboard was probably correlated with this gunshot. The second event was the suicidal gunshot wound to the head, which caused death. Given the extent of blunt force injuries, it was unlikely he could have shot himself after the crash. As the blunt force injuries did not have a typical postmortem appearance, there must have been a very short interval between the fatal gunshot wound and the vehicle's collision with the tree. Most likely, he shot himself as he was driving through the last residential yard or just before the collision occurred.

This unusual suicidal gunshot wound death occurred in a decedent who succeeded in driving with a markedly elevated blood ethanol level (characteristic of chronic alcoholism, which carries an increased risk of suicide). Recent "intolerable" exacerbation of his chronic back pain may also have played a role. The fatal gunshot wound probably followed an attempted suicidal gunshot wound to the neck with a misfire. The correlation of history and scene information with laboratory and autopsy findings allowed reconstruction of the probable chain of events and determination of the cause and manner of death.

### Motor Vehicle Collision, Suicidal Gunshot Wound, Event Reconstruction

## H3 Takayasu Arteritis Involving Coronary Arteries in a Young Black Male: A Case Report and Literature Review

*John Hu, MD, PhD\*, Pinal County Medical Examiner's Office, Florence, AZ*

**Learning Overview:** After attending this presentation, attendees will appreciate typical pathologic features of Takayasu Arteritis (TA) at autopsy, especially in a pediatric patient.

**Impact Statement:** The postmortem diagnosis of TA in autopsy highlights the importance of autopsy as a tool to provide critical pathologic findings to clinicians and facilitate early diagnosis and treatment, which may delay the advancement of the disease and improve prognosis of TA.

**Background:** TA is a rare, chronic, granulomatous, idiopathic vasculitis of large blood vessels. Although it was initially reported mainly in Asia, incidences have been reported worldwide in all ethnicities and age groups. Early diagnosis of TA is challenging in the pediatric population due to its non-specific clinical manifestations and lack of reliable laboratory biomarkers. The differential diagnoses of TA are wide, including other granulomatous vasculitides involving large vessels, sarcoidosis, or tuberculosis or syphilis infections. Clinical intervention often delays advancement of disease and improves prognosis.

**Clinical Presentation:** Presented is an undiagnosed case of TA in a 17-year-old Black man with no known medical history. The patient had complained of non-specific symptoms of malaise, occasional chest pain, and pain the left shoulder since the age of 15. Laboratory tests were remarkable for slightly elevated immunoglobulin. He began to experience palpitations a few weeks prior to his death and intermittent chest pain five days prior to his death. Cardiac troponin-I was elevated. A urine drug screen was negative for drugs of abuse. He was discharged home after a normal echocardiogram study. He was later found unresponsive and was pronounced deceased shortly after arrival at the hospital. Autopsy showed 50% to 70% stenosis of the proximal portions of the right coronary artery and left anterior descending coronary artery with concentrically thickened vascular walls. The intrathoracic aorta, pulmonary trunk and arteries and their primary branches, proximal portion of the brachiocephalic artery, left common carotid artery, and left subclavian artery showed irregularly raised plaques on the intimal surfaces with markedly thickened arterial walls and moderate reduction of luminal sizes. There was no prominent mediastinal lymphadenopathy. There were no gross nodules in the pulmonary parenchyma. Microscopically, extensive infiltrates comprised of lymphocytes and plasma cells were seen in the large vessels, with thickening of entire arterial walls, including the intima and the junction between the tunica media and adventitia. There was disorganization of elastic fibers. Inflammation also involved the vasa vasorum. There were rare granulomata with multinucleated giant cells in the aortic wall. Intimal fibrosis and inflammatory cells infiltrates also involved coronary arteries with reduced lumina and thickening of the adventitia. The myocardium showed multiple foci of wavy myofibers with necrosis and neutrophils. The cause of death was attributed to acute myocardial infarction due to TA involving coronary arteries. Acid-fast bacilli and Grocott-Gomori methenamine silver stains of the involved aorta were unremarkable. A postmortem nasopharyngeal swab was negative for SARS-CoV-2.

**Discussion:** The age of the patient in this case, involvement of mainly large vessels, and overall histologic features are consistent with TA. The clinicopathological features of TA in children and young adults often present challenges to early diagnosis. While early clinical features are often non-specific, malaise, fever, chest pain, pain the left shoulder, and elevated troponin-I of unknown etiology in a young man may warrant further work-up, including detailed physical examination and vascular imaging studies. Myocardial infarction is one of the leading causes of death in patients with TA. Early diagnosis and treatment can delay advancement of disease and improve prognosis.

### Takayasu Arteritis, Autopsy, Myocardial Infarction

## H4 A Fuel Tank Injury In a Fatal Motorcycle Accident

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**Learning Overview:** After attending this presentation, attendees will better understand that some typical lesions can help the forensic pathologist to clarify the dynamics of motorcycle accidents.

**Impact Statement:** This presentation will impact the forensic scientific community by highlighting the importance of injury around the groin area in victims of motorcycle accidents, known as Fuel Tank Injury (FTI) as one of the few characteristic findings.

Vehicle trauma is the leading cause of death in young people. The collision injury mechanism is the cause of direct impact with a fixed object, another motorcycle, or vehicle. FTIs occur when a motorcycle undergoes a violent deceleration following the impact, leading the driver to slide forward against the tank, handlebars, and steering of the vehicle. The driver creates high peak loads between the rider's pelvis and the motorcycle fuel tank, causing abdominal and groin injuries. The 85% of the pelvic injuries sustained by the patient were due to this type of mechanism.<sup>1,2</sup> FTIs are comparatively rare even in drivers of motorcycle accidents because they may occur only from a head-on collision. Some reports showed that the FTI was an evidence of the driver, even though the passengers can also suffer from groin injuries.<sup>3</sup>

A 26-year-old man was riding on a motorcycle with a 600-cc engine (Honda® Hornet®). The motorcycle ran straight into an intersection at high speed and collided with the right-frontal side of a left-turning car. The rider was thrown from the motorcycle onto the roadway by the impact. He was unconscious immediately after the accident and had neither spontaneous breathing nor response on arrival at the emergency department, where he was pronounced dead.

At autopsy, the body showed purple bruising distributed symmetrically over both hips with a partial bowel evisceration through an abdominal lacerated wound, scrotal laceration with exposure of the left testicle, left femur's fracture, and diffuse bruises and abrasions of the head, the trunk, the abdomen, and the four limbs. Multiple rib fractures, thoracic aorta's laceration, intrathoracic herniation of the liver through a diaphragmatic breach, and laceration of the bowel, the spleen, and the liver were also found.

The groin injuries were related to the violent impact with sudden deceleration of the motorcycle, which catapults the rider forward over the saddle. The FTI resulted from gliding and striking of the scrotum and perineum of the rider on the fuel tank during the collision.

For its mechanism, this injury is one of the most useful and important findings of the motorcycle driver and has been described as exclusive to the driver who is sitting behind the fuel tank.<sup>3</sup> The occurrence of groin injuries appears to be dependent on the specific dynamics of the collision and on the impact speed.<sup>4</sup> Therefore, a better knowledge of the FTIs is helpful to understand the dynamics of the event and to discriminate the driver from the passenger, in case this is unknown.

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### Fuel Tank Injury, Groin Injury, Motorcycle Accident

### H5 What Causes the Triad in Diagnosed Abusive Head Trauma (AHT)? Is It the Shaking?

*Thomas Young, MD\*, Heartland Forensic Pathology, LLC, Kansas City, MO*

**Learning Overview:** After attending this presentation, attendees will reconsider diagnosing subdural hemorrhages, retinal hemorrhages, and encephalopathy as AHT.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating through media reports that most cases diagnosed as AHT are neither abusive nor traumatic.

This study utilizes news media reports rather than records generated by physicians. Using media reports instead of doctor-generated reports has advantages, including: (1) obtaining access to unprotected information in the public domain, (2) learning points of view that differ from those that typically diagnose AHT; and (3) learning more about the antecedent past events than the consequent medical outcomes. Disadvantages (which may also be advantages) include: (1) obtaining data from medically untrained individuals (yet this may mitigate bias that physicians who study this problem often carry); (2) encountering frequent bias in media reports (yet such bias frequently favors prosecutors who allege abuse and trauma in AHT court cases and not defendants); and (3) learning unverified information from one source (yet multiple media sources showing similar events are compelling).

Internet searches through [google.com](https://www.google.com) using the words “child abuse shaken baby abusive head trauma” yielded 40 media reports of AHT from September 2, 2020, to August 7, 2021. Reports requiring a paid subscription to access them were excluded. All news reports came from 26 states within the United States.

Victim ages were: 3 weeks to 5 months: 24/40 (24 out of 40 reports); 6 months to 11 months: 5/40; 1 year to 3 years: 6/40; > 3 years: 1/40; age not stated (reported as “infant” or “baby”): 4/40. Children who were unresponsive/non-breathing and transported to a hospital: 32/40; responsive/breathing and transported to a hospital: 5/40 (2 of these for onset of seizures, 1 for lethargy and vomiting, 1 for thermal burns); unresponsive with no mention of a hospital: 2/40; referred to a hospital by an outside doctor: 1/40. Reports with no mention of shaking or head impact from any eyewitness: 22/40; with witnessed accidental trauma: 5/40; with trauma resulting from attempting to revive an unresponsive child: 2/40; with shaking admitted to police: 9/40; with shaking and abusive head impact admitted to police: 2/40; with abusive head impact only admitted to police: 1/40; with abusive trauma (not shaking) witnessed by other(s): 2/40; with shaking or abusive impact spontaneously and independently confessed: 0/40. Reports mentioning skull fractures: 5/40; rib fractures: 4/40; arm fracture: 1/40; ankle fracture: 1/40; numerous fractures not further described: 1/40.

Most infants diagnosed with AHT stop breathing, then are resuscitated but die later in the hospital. Bleeding from a rich dural venous plexus that persists until about age 3, bleeding from hypoxic retinal vessels, and the hypoxic encephalopathy that follows a restoration of blood flow result in the AHT triad. As such, this is a hypoxia problem, not an “abusive” or “head trauma” problem.

Infants who die suddenly and unexpectedly in infancy—“crib death” or “Sudden Infant Death Syndrome (SIDS)”—are typically not successfully resuscitated, so there typically is no triad. Alleged perpetrators may admit shaking or AHT to police officers during an interrogation, whether abuse or head trauma really happened. Those later imprisoned for crimes they did not commit did not recognize the damage done by hypoxia and subsequent resuscitation, and neither did diagnosing doctors.

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#### Abusive Head Trauma, Shaken Baby Syndrome, Triad



## H6 The Forensic Medicolegal System in Qatar

*Ibrahim ElRamahi, MD, Forensic Medicine Center, Doha, Qatar; Khalid BinMarzook, BSc\*, Forensic Medicine Center, Doha, Qatar*

**Learning Overview:** After attending this presentation, attendees will have learned about the different facets of the medicolegal system in Qatar and how it differs from other systems.

**Impact Statement:** This presentation will shed light on the different aspects of the Qatari medicolegal system, which is a combination of the medical examiner system and coroner system that takes into consideration the socio-cultural aspects of the environment it operates within.

Forensic medicine is defined as “the application of medical knowledge to solve legal questions.”<sup>1</sup> It is governed by various legal aspects (that differ from one country to another) that form the medicolegal framework of a country. Appreciating the medicolegal system in Qatar—a system that comprises a combination of both civil and Islamic Shari’a law—can provide a foundation to understanding the medicolegal system in other Arab-Islamic countries.<sup>2</sup> This presentation will set out to do so by expanding on the details of the system in Qatar.

Qatar is a sovereign country, located on the northeastern coast of the Arabian Peninsula in Western Asia. This Arabian, Islamic country is part of the Gulf Cooperation Council (GCC). Its total area is approximately 11,600km<sup>2</sup> and its population is estimated to be approximately 2.7 million, with Qatari nationals making up 400,000 only, while the rest of the population is made up of expats from 110 nations.

Forensic medical services in Qatar are provided to the entire population through the Forensic Medicine Center, which is part of the General Administration of Public Security at the Ministry of Interior. Its services can be broadly categorized into two distinct branches. The first relates to medicolegal death investigations, where autopsies are governed by the Autopsy Law.<sup>3</sup> The other branch relates to medicolegal cases pertaining to the clinical aspect of forensic medicine, which are governed by the Criminal Law and the Manual of Criminal Procedure.<sup>4,5</sup> To further analyze the medicolegal system in Qatar, both of these branches will be detailed. The first branch involves medicolegal death investigation cases that are referred to the center for external examination and/or autopsy in collaboration with Hamad Medical Corporation hospitals. These cases can include sudden death, suicide, medical errors, road traffic collisions, fatal abortions, and criminal deaths, among others, which are then classified as either homicidal, suicidal, or accidental. The second branch involves the clinical aspect of forensic medicine and takes place at the clinics of the Forensic Medicine Center. These include physical assaults, sexual assaults, child abuse, medical liabilities, domestic violence, and work-related injuries, among others.

It should be noted that a forensic pathologist is required to attend all death and crime scenes in order to examine the body at the scene and to determine whether further investigation is required. The district attorney then orders an autopsy and further investigation, including bodily samples and imaging techniques. In criminal cases, virtual autopsy does not suffice and the gold standard of a full autopsy is performed. Prior to the submission of the final report, a preliminary report is completed and submitted within ten days in order to assist in the investigation.

A continental, or multifaceted, medicolegal system is vital due to its adaptability and pragmatism. In a system like the Qatari medicolegal system, roles are clearly defined, and procedures are routine. Indeed, improvements are needed to tackle the deficiencies in some aspects, and more practical protocols are required.

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### Medicolegal Systems, Autopsy, Qatar

## H7 **Railway Fatalities: A Retrospective Analysis of 21 Years**

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**Learning Overview:** After attending this presentation, attendees will have better knowledge regarding railway deaths, including their features and demographics.

**Impact Statement:** This presentation will impact the forensic science community by presenting relevant information related to railway fatalities, including risk factors and injury patterns.

Railway systems are an important part of the infrastructure of the United States. Because of their large mass, collisions involving trains and motor vehicles/pedestrians can be devastating. Severe blunt force injuries generally result from these collisions. In the United States, there have been over 500 railway-related deaths per year over the past 20 years. The majority of the subjects were trespassing on railroad tracks at the time of their death.<sup>1</sup> Previous studies have shown that young, intoxicated males are often the predominant victims of these collisions.<sup>2</sup>

It can be challenging to determine the manner of death in railway fatalities. Medical, social, and psychiatric history, along with scene investigation, autopsy findings, and toxicology results are essential in determining whether the death was accidental or intentional.

In the present study, case files from the El Paso County Medical Examiner's Office database were retrospectively reviewed for railway-related deaths over a 21-year period between January 2000 and December 2020. There were no exclusion criteria. The following records were reviewed: autopsy, investigative, police, and toxicological reports, medical records, and death certificates.

A total of 52 cases were found in the database. The ages of the subjects ranged from 2 to 80 years with a mean age of 35.8 years. There were 40 males (77%) and 12 females (23%). The race was White in 51 cases (including Caucasians and Hispanics—98%), and unknown in 1 case (2%). Forty-nine deaths were due to blunt force injuries (94%), 2 deaths were due to decapitation (4%), and 1 death was due to the traumatic amputation of the upper right extremity (2%). The manner of death was accident in 39 cases (75%), suicide in 7 cases (13.5%), and undetermined in 6 cases (11.5%). There were no homicides.

Evaluating railway-related deaths and their risk factors could shed light on ways to help with prevention of these deaths. An analysis of the cases will be performed, including correlation of location of injuries, time of event, toxicology results, scene investigation, and psychiatric, medical, and social history. A thorough discussion of the results will be presented to the attendees.

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### **Forensic Pathology, Railway Fatalities, Train Accident**

## H8 Pitfalls in Postmortem Computed Tomography

Angela Baldwin, MD, MPH\*, Office of Chief Medical Examiner, New York City, NY; Stacia Semple MD, New York Blood Center Enterprises, New York City, NY

**Learning Overview:** After attending this presentation, attendees will understand that postmortem imaging is best used with caution and always best utilized when in conjunction with autopsy.

**Impact Statement:** This presentation will impact the forensic science community by reinforcing why autopsy is the gold standard for postmortem detection of cause of death and trauma.

**Background:** As imaging techniques and technology improve, postmortem imaging has been increasingly utilized in conjunction with traditional autopsies.<sup>1</sup> Scholing et. al demonstrated agreement of the cause of death between Postmortem Computed Tomography (PMCT) and autopsy varied between 46% and 100%, while the amount of injuries detected on PMCT ranged from 53% to 100% compared with autopsy.<sup>2</sup> The sensitivity of PMCT was increased when combined with postmortem magnetic resonance imaging.<sup>3</sup> Much is at stake when legal/criminal determinations are based on postmortem study. Here is presented one case of suspected pediatric trauma-homicide, further underlying the importance of autopsy in conjunction with imaging studies.

**Case:** A 2-week, 6-day-old term female infant born at 38 weeks gestational age was brought into an emergency department by emergency medical services. One week prior, the infant was evaluated by her pediatrician after multiple episodes of diarrhea, which subsequently resolved two days after the visit. The morning of her death, the infant was reported to be lethargic and was refusing feedings from her mother and grandmother after multiple attempts. In the afternoon, her grandmother retrieved her from where she was sleeping, supine on the couch. The infant had shallow breathing with decreased movement, then became unresponsive in her grandmother's arms. Resuscitative efforts were immediately started. Emergency services arrived and continued resuscitation attempts. Despite all efforts, the infant was pronounced dead upon arrival to the emergency department. A PMCT scan demonstrated displaced-depressed calvarial fracture at the level of the coronal suture, non-displaced right temporal bone fracture, mild depression of the occipital calvarium at the level of the lambdoid sutures bilaterally, and subdural hemorrhage. All other findings were unremarkable. Based on these findings, the child's guardians were questioned by the police. Both denied knowledge of trauma. At autopsy, on external examination, the body was atraumatic, and the scalp without contusions. Internally, overlapping of the parietal skull bones and occipital skull bones at the sutures were noted. The skull and dura were intact. Right parietal intradural patchy petechia as well as parafalcine green-maroon material consistent with aging subacute hemorrhage, also noted. Microscopy revealed rare fibrin-platelet thrombi in the kidneys. Analysis of vitreous humor revealed slight dehydration. Neuropathological examination revealed hypoxic ischemic changes of the brain consistent with sequelae of recent evolving sepsis. Blood cultures failed to grow any organisms, likely due to insufficient sample. Metabolic, toxicologic, molecular, and virology studies were non-contributory. The manner of death was determined to be natural.

**Conclusion:** While postmortem imaging is informative, it is best utilized in conjunction with autopsy as opposed to substitution. Autopsy remains the gold standard.

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### Postmortem Imaging, Autopsy, Infant

## H9 Disseminated Coccidioidomycosis in the Human Skeleton

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**Learning Overview:** Attendees can expect to learn how to recognize the skeletal manifestations of *Coccidioides* fungal infections.

**Impact Statement:** The impact of this presentation will be to inform forensic science practitioners about increasing cases of fungal, bacterial, and infectious processes that go untreated, and result in death.

Coccidiomycosis is a common fungal infection found in the southwestern desert soil of the United States, Mexico, and Central and South America. While 80% of infected individuals are asymptomatic, pulmonary disease is the most common manifestation. In a small percentage of individuals, disseminated lesions are documented and skin, subcutaneous tissue, bone, and brain are targets of the fungus. Reports of Cocci have been increasing since the 1980s and statewide trends reflect this: cases doubled to 10,358 cases from 2014 to 2019, with a surge in 2019. Medical examiner offices have seen an uptick in untreated cases resulting in death. Using two recent cases, this presentation will explore the course of these infections.

An 8-year-old male was found unresponsive by his parents at their shared residence. The mother reported to Emergency Medical Services (EMS) that the child had no illness or trauma, then later added that he vomited the previous night. EMS noted him to have multiple scars and several open wounds, including one bandaged on his forehead, and an open ulcer on his foot. At autopsy, the child was observed to have numerous, irregularly shaped scars all over his body. There were open ulcers on his left foot, right hand, and two on his back. Defects were observed on the forehead, and the back of his head. Two large radiolucent defects were observed radiographically. Upon reflection of the scalp, the occipital defect was filled with soft, pasty, cream-colored material. When the calvarium was removed, a large tumor was observed in the left cerebellar region of the brain. Cultures initially grew *Streptococcus agalacticae*, determined to be a secondary infection. Additionally, the child was in diabetic ketoacidosis due to extreme dehydration.

A 57-year-old female was found decomposed in her residence. Her relevant medical history included lytic left acromion lesion status post-biopsy with pathology demonstrating bone and soft tissue with necrotizing granulomatous inflammation and numerous *Coccidioides* organisms. Laboratory results show positive *Coccidioides immitis* antibody. Medical records review revealed the decedent presented in March 2020 complaining of six months duration pain, weakness, and stiffness in her left shoulder. She also stated she was currently “dealing with valley fever.” At autopsy, postmortem radiographs showed distal acromion with mass-like erosion measuring 3x2x3cm and histology specimens revealed *Coccidioides immitis* spherules in the lung and brain. Family stated that the decedent was normally clean and organized, but that her house looked like it had been ransacked, findings consistent with erratic behavior due to fungus in the brain.

With the advent of novel fungal, bacterial, and viral infections in an era of increased isolation, fear, or lack of access to health care, forensic practitioners are likely to encounter cases more frequently. The Nigerian family in the first case had an aversion to Western medicine and were afraid to leave the home during the COVID-19 pandemic. The father reported that the child suffered from diabetes and had numerous “fatty bumps” and that he (the father) would occasionally incise and drain the contents.

Forensic anthropologists are employed more frequently by forensic pathologists to augment their findings. Recognition, familiarity, and thorough documentation of pathologic conditions that affect bone are crucial for these collaborations. Anthropologists accustomed to seeing lesions in dry bone or, in skeletal or archaeological assemblages, may be confounded when seeing the disease in an active state. Forensic pathologists may be more familiar with the soft tissue changes due to infection, but less familiar with bony lesions produced by the same. Participation by both specialties in these cases will lead to increased familiarity and more comprehensive documentation.

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### Fungal Infections, Coccidioides, Skeletal Lesions

## H10 Scuba Diving Fatalities: A Case Report of a Death Due to a Massive Air Embolism

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**Learning Overview:** After attending this presentation, attendees will better understand the ultimate contribution of postmortem imaging in scuba diving fatalities.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the key role of postmortem Computed Tomography (CT) imaging in a scuba diving death, since air embolism is an uncommon cause of death and air bubbles may disappear during autoptic maneuvers.

A 69-years-old male experienced scuba diver with a history of heart attack went with other experienced divers for a dive to a depth of about 37m. Witnesses said that during the ascent, he signaled that couldn't breathe. After the mandatory stop at the depth of 15m, he made the "OK" sign and continued the ascent. On the surface, he was unconscious and unresponsive. His friends pulled him onto the boat and started Cardiopulmonary Resuscitation (CPR) without resuscitation. Postmortem ascertainments were set.

Postmortem total body CT showed air-equivalent attenuation values in several arteries, including anterior and middle cerebral artery as well as vertebrobasilar system (Cerebral Arterial Gas Embolism [CAGE]), supraaortic trunks, cardiac cavities, coronary arteries, and arterial-venous thoraco-abdominal and extremities circulation. No signs of pneumothorax, pneumomediastinum, or subcutaneous emphysema were found.

In the light of radiological findings, the autopsy was set with special techniques to highlight air bubbles in cerebral arteries and sample the gas from heart cavities. Gas inclusions were detected in all arteries of the circle of Willis. The heart floated in water and its cavities were filled with microbubbles. The histological findings excluded that bubbles were a postmortem artifact. The cause of death was identified in massive gas embolism due to decompression sickness.

Technical ascertainment established that all decompression procedures had been respected and diving equipment was well functioning; however, the sintered filter was unclean and the Venturi effect deactivated, resulting in an overall decrease of oxygen rate.

According to annual diving reports, the primary cause of death in diving fatalities is drowning, followed by heart disease. Arterial Gas Embolism (AGE) and severe Decompression Sickness (DCS) represent a small percentage of fatal cases. They are caused by rapid decompression, which leads to the formation of gas bubbles in the blood. They may also depend on diving equipment malfunction.

The autoptic diagnosis of air embolism may be difficult since air bubbles usually disappear during organ extraction maneuvers. In the present case, medical history, witnesses' account, and ascertainment on the equipment placed the differential diagnosis with a cardiac death. However, it was postmortem CT that played a fundamental role in suggesting the presence of arterial gas embolism.

In this way forensic radiology represented a valuable tool to guide the subsequent autoptic procedure and indicate the correct technical approach according to the diagnostic suspect. Postmortem CT confirmed its essential diagnostic significance in scuba diving fatalities, especially providing relevant additional information to direct autopsy and support pathological findings.

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### Scuba Diving, Air Embolism, Postmortem Computed Tomography

## H11 FVIII Expression to Differentiate Vital Hemorrhages From Postmortem Artifacts

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**Learning Overview:** The goal of this presentation is to show how the search for vitality markers of soft tissue injuries allowed the forensic pathologist to be moved into a wide spectrum of techniques to reach the correct diagnosis. In this context, immunohistochemical analyses provide evidence that, integrated together, can strongly corroborate the diagnosis of vitality, thus making them mandatory for forensic pathologists.

**Impact Statement:** This presentation will impact the forensic pathologists community by highlighting the need to establish a pattern of markers that should be routinely performed for the diagnosis of viability of soft tissue lesions, including Factor VIII (FVIII).

Wound age assessment is one of the most challenging issues in forensic pathology. While Red Blood Cell (RBC) infiltration is classically considered a sign of a vital reaction, several studies have shown that extravasation of blood cells can occur even after death and cannot be used as a reliable marker in the diagnosis of wound viability. Indeed, macroscopic artifacts may be related to postmortem Prinsloo-Gordon hemorrhages or hypostatic infiltrations, injuries related to resuscitation, or from macro- and micro-fauna alterations. On the other hand, in the literature, Immunohistochemistry (IHC), an important diagnostic tool in anatomical and surgical pathology, is used less frequently in forensic pathology. Tissue degradation due to postmortem decomposition is believed to be an important limiting factor, although it is unclear what impact this degradation actually has on the validity of IHC staining. According to some authors, autolysis would induce a higher risk of false positives; according to others, the advancement of decomposition would involve a higher risk of false negatives.

In this context, this study aimed: (1) to underpin the importance of histological examination to precisely qualify lesions; (2) to test an immunohistochemical biomarker and, subsequently, a pattern capable of diagnosing vital injuries from postmortem artifacts.

Skin samples collected from selected subjects who died from trauma were analyzed and compared with those of the control. Moreover, as positive control, skin specimens resulted positive to immunohistochemical reaction with antibody anti-CD15 and anti-tryptase.

The results of the present study confirmed the importance of histology as a fundamental tool in order to produce evidence for the diagnosis of the identification of the lesions and, therefore, of the cause of death. Furthermore, different cellular and cytokine expression patterns are associated with antibody positivity, compared to the control case. Furthermore, it is interesting to note the behavior of the immunohistochemical reaction using anti-FVIII antibodies.

Based on the expression of FVIII in wound hemorrhage and data collected from the literature review analysis, although preliminary and limited in number, it seems to suggest the utility of this marker in combinations of multiple markers to achieve vitality diagnosis.

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### FVIII, Hemorrhage, Vitality

### **H12 Sudden Death After Contrast Media Administration—Not Always Anaphylaxis: A Workflow for Diagnosis**

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**WITHDRAWN**

### H13 Evaluating Trends and Demographics of Accidental Drug Deaths in Harris County, Texas, From 2016 to 2020

*Varsha Podduturi, MD\**, Harris County Institute of Forensic Sciences, Houston, TX; *Namrata Walia, MD, MHA*, Baylor College of Medicine, Houston, TX and UT Health School of Public Health, Houston, TX; *Daryl Shorter, MD*, The Menninger Clinic, Houston, TX

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**Learning Overview:** After attending this presentation, attendees will understand the drug trends associated with the most commonly used substances and decedent demographics of accidental drug deaths from 2016 to 2020 in Harris County, TX (which includes the city of Houston).

**Impact Statement:** This presentation will impact the forensic science community by examining drug trends and the demographics of the population involved within Harris County, TX.

Cases classified as accidents represented 32% to 40% of all the total cases between 2016 and 2020. A significant number of the accidental deaths were related to drug intoxication (ranging from 32%-51%), followed by motor vehicle crashes (ranging from 29-33%). The electronic database from the Harris County Institute of Forensic Sciences (HCIFS) in Houston, TX, was searched for all accidental drug deaths between 2016 and 2020. The following data was collected: cause of death, age, sex, and ethnicity. Each case was evaluated for the substances listed in the cause of death and each intoxicant was subsequently coded in a database that categorized substances in general categories based on its designation within the *International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10)* system, such as narcotics, psychodysleptics, and sedative-hypnotic drugs, and further subcategorized into more specific categories with specific substance names.

Evaluation of the data showed the total number of accidental drug deaths in Harris County increased from 429 in 2016 to 989 in 2020, which represents an increase of 130%. White males (average of 36.23%), followed by Black males (average of 18.46%) and Hispanic males (average of 17.03%,) were the most impacted by accidental drug intoxications. Among females, White females (15.3%) were more prevalent than Black (7.65%) and Hispanic (3.26%) females.

Hispanic females had the least number of intoxication deaths among the decedent population with 2% or more of the cases. Hispanic males and White females had decreased numbers of drug deaths within their populations. Others (2.07%), including Asians and American Indians, continued to be the least affected.

A review of the data indicated that cocaine is the most frequent intoxicant in fatalities. Accidental drug deaths may involve multiple intoxicants; therefore, a single case may be included in more than one category when describing the number of drug deaths involving specific substances. In the order of prevalence, cocaine, methamphetamine, benzodiazepines, and prescription opioids were commonly detected. Fentanyl and analogs were found in less than 5% of the cases in 2016. However, they were reported in over 30% of cases in 2020. Similarly, methamphetamine was reported in 56 cases in 2016 (13% of cases), compared to 352 cases in 2020 (35.63% of total cases), representing a 174% increase. The use of benzodiazepines has slowly increased over the five-year period reviewed. Conversely, the numbers of cases involving prescription opioids gradually decreased as other drugs such as methamphetamines and fentanyl became more prevalent.

Harris County has continuously experienced record highs among its intoxication deaths, especially within the White male population. The increasing numbers of accidental drug deaths, specifically those related to methamphetamine and fentanyl, require attention. This information can be useful for public health campaigns for targeting groups most at risk. Additionally, the rise of fentanyl analogs emphasizes how critical it is for toxicology laboratories to be able to provide testing to detect these emerging substances.

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#### Toxicology, Drug Deaths, Drug Trends



## H14 An Unexpected and Rare Cause of Child Death: A Large Obstructing Fecaloma

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**Learning Overview:** The aim of this paper is to highlight the importance of the use of immunohistochemical investigation in complex cases. The presented case is a unique case of child sudden death due to an obstructing fecal impaction.

**Impact Statement:** This presentation will impact the forensic community, showing that as for the cause of death, the systematic application of immunohistochemical investigation is the only tool to obtain more information, especially in this unique case of death by fecaloma. In fact, in the event of sudden death, in the absence of anamnestic and clinical information, the pathologist cannot identify the exact mechanism of death only with the autopsy examination. In this perspective, immunohistochemistry highlights alterations not noticeable by macroscopic or histological observation.

**Text:** A 9-year-old boy started complaining of severe abdominal pain during the night. The mother of the child called the emergency service, explaining that the child was suffering from intense abdominal pain and diarrhea. She was invited by the doctor to hydrate only the child and to wait until morning. The boy continued to suffer and, at last, he suddenly died at 6.00 a.m. The parents reported that the child had no symptoms in the previous days and did not suffer from any illness. So, being the cause of death was unclear, an autopsy was performed. On external examination, the body did not show external injuries. The abdomen was distended and entirely affected by the putrefactive green stain. Opening the peritoneal cavity, there was a massive expansion of the intestinal loops without apparent necrosis. The intestine was isolated and opened on his anti-mesenteric side. In the lumen of the rectum-sigma, about 18cm from the anus, there was a large hard fecal mass, measuring 7x5.5x4cm. The fecal mass obstructed the lumen. The whole gastrointestinal system contained fecaloid material, while the esophagus was full of blackish fecal-like material. The organs did not show macroscopic alteration. The toxicological tests carried out on blood and urine were negative. The study of the samples of the organs, stained with hematoxylin-eosin, revealed visceral stasis, massive pulmonary edema, and interfascicular edema of the myocardium. The histological examination of the intestinal wall revealed no necrosis. Immunohistochemical analysis was performed using antibodies anti-CD68, MCP-1, TNF $\alpha$ , and COX2. The intestinal wall showed intense positivity for CD68 antibodies on the mucosal side. The other markers were more noticeable on the serous side. The immunohistochemical positivity thus demonstrated the presence of inflammation of the intestinal wall. The inflamed intestinal wall leads to a hydro electrolytic imbalance due to the lack of reabsorption of fluids. These mechanisms contributed to the death of the boy. The sudden death of the child was due to acute heart failure secondary to a hydro electrolytic imbalance caused by intestinal obstruction.

Bowel obstruction is a mechanical obstruction that interrupts the flow of intestinal contents, resulting in proximal bowel dilatation and distal bowel decompression. The progression of obstructions increases the degree of dilatation, causing bowel wall ischemia, necrosis, and even perforation with associated peritonitis and sepsis, necessitating urgent surgery.<sup>1</sup> Causes of large bowel obstruction in children are helminths infection, bezoars (solid masses of indigestible material), congenital abnormal bands, intestinal malrotation, Hirschsprung disease, Meckel's diverticulum, etc.<sup>1,2</sup>

In the presented case, the cause of the bowel obstruction was a large fecaloma. A Fecal Impaction (FI) is a large mass of compacted stool that is impossible to evacuate spontaneously.<sup>3</sup> The distal colon and rectum are the most common sites for fecalomas.<sup>4</sup> This condition is uncommon in children, the majority of reported cases are in elderly or psychiatric patients.<sup>5</sup> There are multiple causes of fecaloma formation, but in the described case, the cause is unknown as the parents did not report a history of chronic constipation or other diseases.<sup>6,7</sup>

Sudden unexpected death depending on gastrointestinal diseases is not common, especially compared to other conditions such as cardiovascular conditions or infection.<sup>8</sup> In literature, there are not reported cases of infant death due to fecal impaction. Generally, parents describe a history of chronic constipation and abdominal pain. The absence of anamnestic information did not guide the diagnosis of death. The autopsy was crucial to finding the fecal impaction.

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### Fecaloma, Sudden Child Death, Immunohistochemistry

## H15 Streptococcal Toxic Shock Syndrome: A Cautionary Tale

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**Learning Overview:** Attendees can expect to learn about the signs, symptoms, autopsy findings, and suggested autopsy workup of Streptococcal Toxic Shock Syndrome (STSS).

**Impact Statement:** This presentation will remind the learner of the existence of STSS, its potential pathogens, complications, and clinical scenario. The autopsy or forensic pathologist will be aware of this disease and its postmortem workup, which may aid in its detection and, where appropriate, notification of public health agencies.

**Background:** TSS is an acute, severe, toxin-mediated illness resulting from superantigen production by *Staphylococcus aureus* or *Beta-hemolytic Streptococcus*, resulting in rapid onset shock and organ failure.<sup>1,2</sup> Risk factors include skin wounds, viral infection, or other minor trauma (e.g., sprains, bruises). Clinically, a prodrome of fever, myalgia, chills, nausea, vomiting, and diarrhea may precede hypotension and multisystem organ failure by a period of hours to days.<sup>3</sup> Evidence of deep soft tissue involvement, including necrotizing fasciitis or myonecrosis, may appear within 24–72 hours.<sup>4</sup> The incidence of STSS is increasing in North America and Europe, with a mortality rate of 5-10%.<sup>1,5</sup>

**Case Report:** The patient was a previously healthy adolescent female who presented to the emergency department following several days of cough, flank pain, sore throat, nausea, vomiting, and worsening dyspnea and orthopnea. Testing for COVID-19 was negative. She had bilateral pleural effusions, respiratory failure requiring immediate intubation, and acute kidney injury requiring dialysis. Her course was further complicated by development of Acute Respiratory Distress Syndrome (ARDS), necrosis of a hand, pancytopenia, purpura fulminans, Disseminated Intravascular Coagulation (DIC), and septic shock requiring multiple vasopressors. Blood cultures grew Group G Streptococcus, and a diagnosis of STSS was rendered. Despite treatment with antimicrobials and Intravenous Immunoglobulin (IVIg), her status declined and she died.

**Autopsy Findings:** External examination showed soft tissue necrosis of the hand and purpura fulminans. Microscopic examination of the lungs showed necrotizing pneumonia and Diffuse Alveolar Damage (DAD) with hyaline membrane formation with invasive fungal organisms. Similar fungal organisms were present in sections of the trachea. Other organs showed evidence of ischemia and shock: acute tubular necrosis with cellular casts in the kidneys, centrilobular (zone 3) hepatocyte necrosis of the liver, multiple microscopic infarcts with associated microcalcifications of the myocardium, and ischemic changes in the hippocampal neurons.

Tissue cultures taken of the lung and spleen grew *Candida albicans*, which was morphologically consistent with the invasive fungal organisms noted in the lungs and trachea histologically. Bacterial cultures of the lung and spleen showed no growth of aerobic, anaerobic, or mycobacterial organisms. A viral culture of the spleen was negative. Blood cultures were not performed due to the patient's antemortem positive cultures and treatment with antimicrobials.

**Discussion:** The causative agent of this patient's case was identified as Group G Streptococcus, which, while not as common as Group A Streptococcus in STSS, has nonetheless been described previously.<sup>2</sup> The invasive *Candida albicans* was attributed to an opportunistic takeover of normal flora in the setting of sepsis and severe immunocompromise. A nidus of infection was not identified, though this is not necessary for diagnosis and, in many cases, may never be definitively elucidated.<sup>5</sup>

While this patient's case followed the "textbook" STSS sequence of non-specific prodromal symptoms rapidly devolving into septic shock, multisystem organ failure, and DIC with soft tissue necrosis and purpura fulminans, STSS may lack soft tissue involvement and instead mimic other pathologies, including pneumonia, myocardial infarction, peritonitis, or pelvic inflammatory disease.<sup>4</sup> Cutaneous lesions may also resemble patterned or inflicted trauma.<sup>6</sup> The fast and furious onset of STSS has the potential to be lethal before a definitive diagnosis is obtained, or before medical care may be sought. Forensic and hospital autopsy pathologists must therefore maintain a high index of suspicion for TSS in the setting of sepsis, with a low threshold for obtaining blood and tissue cultures, performing histologic examination, and, where necessary, notifying public health agencies.<sup>7</sup>

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### Toxic Shock Syndrome, Streptococcus, Sepsis

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## H16 Air Embolism: An Important Autopsy Finding in Gunshot Wounds of the Head Deaths

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the frequency, significance, and methods for detecting air embolism in deaths due to a gunshot wound of the head.

**Impact Statement:** This presentation will impact the forensic science community by emphasizing the significance of identifying air embolism in deaths due to gunshot wounds of the head, including its possible contribution to the mechanism of death and the opportunity it presents for educational purposes and maintenance of competency.

Air embolism occurs as a result of air entering the blood circulation. It is reported that when greater than 50ml air is introduced into the circulation, it may result in pulmonary outflow obstruction.<sup>1</sup> When a large amount of air enters the circulation, it may lodge in the right side of the heart, causing diminished and obstructed outflow of blood from the pulmonary artery, which may contribute to the mechanism of death. The effect of the embolism is determined by the total volume, the speed, and the location of the air embolism.<sup>2</sup> In a forensic setting, its presence may be investigated in deaths involving open wounds of the neck, chest trauma, childbirth, diving, and certain medical procedures. However, it can also be caused by open head injuries with exposure of the dural sinuses.

Air embolism can be detected by postmortem radiography and can be confirmed by multiple postmortem methods, including needle aspiration from the right heart chambers using a water-filled syringe. Air bubbles emerging from the heart chambers are considered a positive finding.

Gunshot wounds of the head are a common cause of open head injuries in a medical examiner or coroner office setting. This study reports the occurrence rate of air embolism detected by postmortem radiography in deaths due to a gunshot wound of the head, reviews methods to identify the air embolism, and discusses its significance in forensic practice.

A Cook County Medical Examiner Office database search of the 7/1/2020 to 7/1/2021 cases resulted 154 deaths due to a gunshot wound of the head. Cases that had thoracic organ donation and signs of decomposition by visual inspection were excluded from the study; 145 cases were included in the statistical analysis. Anterior-posterior chest X-ray images were reviewed. Air embolism was identified by a radiolucent area in the right heart, typically involving the superior vena cava, right atrium, right ventricle, and in some cases, extending to the pulmonary artery. Thirty of the 145 gunshot wound of the head cases (20.7%) had radiographic evidence of air embolism. Among these cases, only one had craniotomy, four had intravenous and/or interosseous infusion catheters, and the remaining cases had no invasive medical intervention or no medical intervention at all. All decedents included in the statistical analysis were pronounced within 24 hours after being shot, and the examinations were completed by one day after pronouncement. The control group included 2 gunshot wound of the abdomen cases, 11 gunshot wound of the chest cases, and 18 multiple gunshot wounds cases without head injury. The control cases were selected using the same exclusion criteria (no decomposition, no thoracic organ donation). In addition, cases with injury to the heart were excluded. None of the 31 control cases (0%) had radiographic evidence of air embolism.

**Conclusion:** Air embolism is occasionally observed in head trauma, including deaths due to gunshot wounds of the head. Identification of air embolism on postmortem radiography prior to autopsy is essential to be able to confirm at the time of examination. Familiarization with the phenomenon and its detection methods facilitates forensic pathologists in case documentation and potential forensic implications.

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### Air Embolism, Gunshot, Head

## H17 Anomalous Ligature Materials and Means in Hangings: A Report of Ten Cases

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**Learning Overview:** The purpose of this presentation is to show how the forensic pathologist faces a large spectrum of ligature materials and improvised means employed to commit hanging. It can cause singular ligature marks, thus it is relevant to determine the compatibility between marks and materials/means through an attentive and comparative analysis; the ligature mark vitality through histological and immune-histochemical examinations must be assessed too.

**Impact Statement:** This presentation will affect forensic pathologists by bringing attention to a complete approach in studying hangings that occurred in anomalous circumstances/performed with unusual means through a careful examination, including a comparative analysis of the ligature mark both with materials and means employed.

Hanging is one of the most common methods used to commit suicide. Homicidal and accidental hangings are rare. Hanging consists of the complete or partial body suspension with a ligature around the neck. Ligature materials can be various; victims may use anything available at that moment. So, in some cases, the victims use unusual means to commit suicide.

The present study is a review of the autopsy reports of ten suicidal hangings observed in the period 2010–2021 at the Department of Forensic Sciences of the University of Foggia, in which the circumstances and the ligature means were different than usual (i.e., ropes, laces, etc.).

Regarding the unusual means used for the hanging, the review pointed out the case of an 82-year-old man that partially suspended himself by a nasal cannula fixed to a metal hook to his kitchen's ceiling; a 24-year-old man found lifeless in his prison cell, hanged with a noose made by cutting a cotton T-shirt into a narrow strip, hooked to a metal window bar; a 26-year-old found hanging with a horse bridle to a ceiling beam of a stable; a 45-year-old found incompletely suspended by two tied shoelaces to his bedroom's door.

Regarding the circumstances in which the hanging happened, the review includes the case of an under-24h-visual-surveillance 46-year-old man, found lifeless in his prison cell, partially hanging with his pant drawstring to the bathroom's window grate; a 50-year-old drug-addict, found partially suspended by two tied bathrobe belts to his room's radiator in a recovery center; a 9-year-old child incompletely suspended with his neck inside the handle of an olive sack; a 30-year-old hanged with the shoulder strap of a gym bag hooked to the top of an emergency call box on a highway; a 47-year-old drug addict, partially hanged with a waist belt to a French window in his bedroom; and a 74-year-old, hospitalized in a psychiatric center, incompletely suspended by a towel to the bathroom's radiator.

In many of these cases, the ligature means had been already removed when the pathologist performed the crime scene investigation because the relatives attempted to rescue the victim by removing the ligature from around the neck. Considering the peculiar materials used to commit hangings, it has been necessary to compare them with the injuries and verify the circumstances of death to exclude different events than suicides.

The study highlights that analysis of the ligature's mark and the histological and immunohistochemical examinations can help to determine the vitality of injuries and the compatibility with the alleged means of hanging. In the ten cases, these elements have been fundamental to reach a differential diagnosis between suicide, homicide, and accident.

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### Anomalous Ligature Means, Hangings, Suicides

## H18 The Use of Forensic Radiology in the Assessment of the Emergency Department Presentation of Near-Hanging Victims in the United Kingdom

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**Learning Overview:** After attending this presentation, attendees can expect to better understand the use of forensic radiology in assessing victims of near-hangings that present to hospital emergency departments with potentially significant head and neck trauma. Attendees will gain insight into the use and varying reliability of easily measurable parameters identified upon presentation in near-hanging victims for management improvement purposes; their use in mortality/morbidity prediction; and acquire important information about hanging as seen through radiology.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness on the variability of presentations of near-hanging victims, which hinders the development of management and investigation guidelines for such cases. Additionally, the original casework presented herein will add to what is currently known about near-hanging victim presentations and prognoses to help shape such guidelines and highlight the importance of cervical radiology in assessing the gravity of injury sustained.

Hanging is commonly encountered in the forensic setting, where the conventional autopsy and postmortem radiology serve to distinguish from the pattern(s) of injury, suicide, and accidental death from homicide. Hanging is the most commonly employed method of suicide for both men and women in England and Wales, reflecting a global trend.<sup>1,2</sup> The fatality of hanging is high, but individuals who survive to hospital are termed “near-hanging” victims and may present with numerous signs varying across a wide range of severities.<sup>3</sup> While many of these patients will leave the hospital alive and well, in-patient deaths in this population are not uncommon, and others who survive may only do so with a neuropsychological disability. Radiology is a critical tool for the successful investigation and management of these patients.

However, the observed versatility of injury and possible outcomes mean robust guidelines for the treatment of near-hanging victims have yet to be established.<sup>4</sup> The prediction of mortality and morbidity of near-hanging victims presenting to hospital is important for the patients, but also for their families and friends.

Currently, Glasgow Coma Scale (GCS) score and cardiopulmonary arrest prior to being admitted are the only presenting parameters (excluding what is forensically known about the scene, such as hanging time and drop height) that have been identified in the literature as predictors of mortality. No accurate predictors of morbidity have been established for near-hanging victims.<sup>5</sup>

**Objective:** This study assesses if presenting parameters (age, sex, presence of a ligature mark, and earliest GCS score) can constitute adequate morbidity/mortality predictors for maximizing the triage, radiological investigation, and medical management of near-hanging victims.

**Methods:** Computed Tomography (CT) scans of 21 near-hanging victims presenting to hospitals in the United Kingdom were subjected to forensic radiology analysis. Raw data was processed using SPSS to establish the incidence of injury, mortality, and population demographics.

**Results:** No presenting parameters were deemed dependable predictors of radiologically identifiable injury. A GCS score  $\leq 3$  was statistically significant ( $p=0.044$ ) and associated with higher mortality. Overall trends indicated increasing age was correlated with an increased risk of injury and death.

**Conclusions:** Findings of this present study confirm information previously reported in the literature. As presenting parameters are not sufficiently reliable to determine the extent of injury and the prognosis of near-hanging patients, early investigation with CT scanning is recommended in all patients to prevent overlooking potentially devastating injury.

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### Radiology, Near-Hanging Victims, Emergency Department

### **H19 Wound Vitality and the Estimation of Wound- Age Production: New Frontiers Through Immunohistochemistry Investigation**

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**Learning Overview:** Evaluation of the vitality of soft tissues injuries still represents a challenging topic for forensic pathologists, who aim to estimate wound-age production. This purpose is even harder in cases of corpses in advanced decomposition states. Immunohistochemical techniques are becoming an indispensable aid when the macroscopic investigation is not determining.

**Impact Statement:** This presentation will impact attendees by showing that the systematic application of the tested techniques could help forensic pathologists to get diagnostic elements in cases, only observed in forensic practice, in which biological materials are not well preserved, but it is mandatory to establish a cause of death and/or the timing of the production of wounds.

This pilot study aims to apply the immunohistochemistry investigations to evaluate the vitality of the lesion by using monoclonal antibodies against CD15 (produced by leukocytes, especially in neutrophils), IL-15 (cytokine secreted by mononuclear phagocytes and some other cells following infection by a virus), tryptase (an enzyme released by mast cells), and glycophorin A (red blood cell transmembrane proteins). Literature data indicate that the quantification of their expression can estimate the age production of a wound. Moreover, in this study, a comparison between the Hematoxylin-Eosin (H&E) staining and immunohistochemistry techniques is also provided to evaluate their potential in identifying “vital” processes, above all in putrefied soft tissues.

The study examined 12 cases in which autopsies were performed some days after death because the corpses were not found immediately. In all these cases, causes/circumstances of death were uncertain (natural vs. unnatural death), and all bodies had lesions and/or mutilations with doubtful relevance regarding the cause of death. In many cases, the putrefaction process was so advanced as to not allow the distinction between vital or not vital injury. Many samples were taken from the edges of the lesions, both in the soft tissues and bones. The samples were fixed in 10% formalin and from each sample were obtained at least five slides. The slides of every case were stained with H&E, CD 15, IL 15, tryptase, and glycophorin A, then observed on the optical microscope. Samples from the bodies’ areas without injuries were taken and stained with the same techniques.

The preliminary results suggest that the immunohistochemical stainings should be routinely applied in cases of corpses with advanced putrefaction phenomenon; even if the radiological investigation is performed, the macroscopic examination is inconclusive, and the H&E staining is not reliable.

Though certainly not conclusive, the experimental application carried out in this study suggests that the use of monoclonal antibodies anti-CD15, anti-IL-15, anti-tryptase, and anti-glycophorin A may be important to diagnose the vitality/not vitality of lesions and to estimate the timing of the production of a wound.

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#### **Wound Vitality, Anti-CD15, Anti-Glycophorin A**

## H20 A Forensic Approach to an Italian Mass Disaster: The Collapse of the Morandi Bridge

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of involving the forensic science community in mass disaster management.

**Impact Statement:** This presentation will impact the forensic science community by presenting the organization of the morgue following the mass fatality of the Morandi Bridge collapse.

Mass disasters are generally understood as crisis situations that go beyond a community's ability to recover. They are usually unpredictable and unexpected situations demanding a multidisciplinary and well-coordinated approach. On August 14, 2018, at 11:36 a.m., a 690ft section of the Genoa Morandi Bridge collapsed. Thirty-three vehicles traveling on it fell from an height of 130ft into the river below and the surrounding industrial area. Fifty-eight people were directly involved in the fall: 43 died and 15 were wounded. The death toll was: 29 Italian citizens, 4 French, 3 Chileans, 2 Albanians, 1 Colombian, 1 Jamaican, 1 Peruvian, and 1 Romanian. During the two days following the collapse, the 43 bodies were found either in the fallen vehicles or in the industrial area below the bridge and subsequently taken to the town morgue of Genoa. Immediately after the event, all the medical staff of the Departmental Section of Legal and Forensic Medicine of Genoa cooperated with the Civil Protection Agency, psychologists, and the public prosecutor.

For the victims' identification, the International Criminal Police Organization (INTERPOL) Disaster Victim Identification (DVI) process was used. A postmortem team photographed the victims, collected DNA samples, and took notes of their personal belongings, general physical characteristics, and distinguishing features. Collaborating with the Civil Protection Agency, in charge of the rescue, was fundamental for determining in which vehicle each victim was found and who the passengers of each vehicle were. At the same time, an antemortem team interviewed the victims' relatives who arrived at the morgue. Finally, antemortem and postmortem data were compared to identify potential matches between a found body and a missing person.

Despite the height of fall, all the victims preserved strong identification marks; this allowed the use of visual identification to confidently confirm the identity of all the victims without awaiting the DNA comparison results. The victims were identified in less than 24 hours from arrival at the town morgue. After receiving the authorization of the public persecutor, all bodies underwent a forensic examination to identify the cause of death: in 29 cases, multiple traumatic severe lesions were found and the cause of death was attributed to a polytrauma; in 12 cases, traumatic injuries had forced brain tissue out from the skull, causing a severe cranioencephalic traumatism; and finally, in 2 cases, the cause was found to be cervical spine fractures with wide spinal cord involvement.

Seven months after the collapse, in accordance with Italian law, 37 people were evaluated to identify personal injuries related to the event: 15 people were directly involved in the fall and reported predominantly physical damages, while the remaining 22 complained only of psychological damages due to having seen the bridge fall. Personal injuries were detected in 34 cases. After the Morandi Bridge collapse, authorities carried out an extensive investigation that showed the responsibilities of different companies involved in the bridge maintenance.

This presentation brings to light that the combination of a correct forensic approach and the teamwork of different professional profiles is essential to manage mass fatality accidents, both for purposes of criminal investigation and as a moral obligation vis-a-vis the victim's family members. The Morandi collapse also shows that every mass disaster has unique features and requires a tailored approach; this is to be developed through experience, pre-planning, and continuous training.

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### Forensic Pathology, Disaster Victim Identification, Biological Damage Evaluation

## H21 The Death of a Professional Scuba Diver Due to Gas Embolism: An Autopsy Case

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**Learning Overview:** After attending this presentation, attendees will have meaningful insights on radiological and pathological features and autoptic findings in cases of decompression sickness.

**Impact Statement:** This presentation will impact the forensic science community by providing striking visuals and videos of histopathological, radiological, and macroscopic findings of air embolism within coronary and cerebral vessels.

Gas embolism or decompression sickness is a result of rapid decompression, which gives rise to bubble formation within tissues from dissolved inert gas. When pulmonary barotrauma occurs due to pressure gradient, intra-alveolar gas can penetrate to the intravascular space of pulmonary vessels, then to the systemic circulation through the pulmonary veins. Death can occur either by gas embolism in cerebral or coronary circulations or by cardiac failure due to intraventricular “airlock.”

In this case report, a 37-year-old professional military diver, who lost consciousness during ascent from a deep dive on a training session and died after being unresponsive to cardiopulmonary resuscitation, is presented. After the arrival of the deceased to the morgue, an X-ray of the chest revealed air within cardiac chambers, the carotid artery, and cerebral vessels. Consequently, an autopsy investigation was carried out to establish the exact cause of death. The gross examination of the brain revealed very clear and striking visuals of air bubbles within superficial cerebral veins. Furthermore, the air bubbles were present in superficial cardiac vessels, as well.

Photographic and video documentation was made, and appropriate samples were taken for toxicological and histopathological analysis. Microscopic examinations confirmed the air presence within the cerebral and cardiac vessels, and the pulmonary barotrauma.

Cerebral arterial gas embolism is more likely to happen in scuba divers because of the vertical position of the person during ascension and the tendency of the gas bubbles that are forming within the circulation to float. Cerebral arterial gas embolism presents with loss of consciousness within minutes after the rapid ascension and cardiac arrest that is unresponsive to cardiopulmonary resuscitation. A controlled ascent, therefore, is of utmost importance to avoid rapid decompression “off-gassing” (gas pass from the solution into the vessel lumens because of pressure changes).

The presented case is an excellent visual example of decompression sickness. The findings identified during the investigations carried out, together with the presentation of the condition, made it possible to identify the cause of death as arterial gas embolism due to pulmonary barotrauma. Conclusively, this case highlights the importance of having a systematic approach to scuba-diving deaths to determine the exact cause of death, using available radiological properties, and choosing the appropriate autopsy technique. The forensic practitioner who conducts the autopsy for a scuba-diving fatality should have knowledge and experience about the physiopathology of barotraumas.

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### Gas Embolism, Decompression Sickness, Autopsy



## H22 An Anomalous Origin of Left Coronary Artery With Retro-Aortic Course and Sudden Cardiac Death

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**Learning Overview:** After attending this presentation, attendees will have learned that a comprehensive autopsy, including expert cardio-pathological examination, is of the utmost importance in cases of Sudden Cardiac Death (SCD), especially when cardiac anomalies are classified as uncertain cause of death.

**Impact Statement:** This presentation will impact the forensic science community because medical malpractice is often claimed in cases of unexplained SCD in young people. Issues related to cardiovascular screening of young athletes and improper resuscitation will be also discussed.

**Introduction:** Congenital anomalies of coronary arteries are often asymptomatic and may cause death with different degrees of certainty (i.e., “certain, highly probable, or uncertain”). While the anomalous origin of Left Main Coronary Artery (LMCA) from the opposite sinus with inter-arterial course has been reported as a “highly probable” substrate of SCD, when the anomalous origin of LMCA is followed by a retro-aortic course, this association has been reported as “uncertain.”

**Case Report:** An 8-year-old boy, in apparent good health, suddenly fell on the playground during a football training. His two Basic Life Support Defibrillation (BLSD) -trained coaches promptly performed resuscitation with the help of the emergency medical service telephonic suggestions.

Unfortunately, although an automated external defibrillator was available at the training site (as recommended from the Federation Internationale de Football Association [FIFA]), it was not used. After about 15 minutes, the medical staff arrived on site and declared the boy dead on arrival. At autopsy, LMCA anomalous origin from right coronary sinus of Valsalva with a separate ostium followed by retro-aortic course was observed. In addition, the left coronary artery displayed separate coronary ostium, acute angle takeoff from the aorta, and hypoplasia of its main branches. Microscopic examination revealed multiple foci of recent (days-weeks) ischemic lesions in inflammatory reparative phase, associated with mild multifocal lymphocytic myocarditis.

**Discussion:** Anomalous origin of the LMCA from the contralateral Valsalva sinus without inter-arterial course is infrequently diagnosed in children and, based on the available data, have been reported as an “uncertain” substrate of SCD. In the present case, the additional described alterations probably concurred to reduce the blood flow in the anomalous vessel, resulting in myocardial ischemia foci and in SCD during exercise. It is also possible that the superimposed mild lymphocytic myocarditis played a role. In this specific case, SCD occurred in a young athlete, despite regular cardiovascular screening tests, including Electrocardiogram (ECG) and echocardiography, which were unsuitable to diagnose such coronary artery anomalies. The forensic evaluation about the cause and the manner of death considered also the modalities of the resuscitation attempts and the claimed malpractice, as often occurs in case of sudden unexpected death in young athletes. Anomalies of coronary artery origin should require case-by-case evaluation, including clinical history, circumstances of death, and results of detailed cardio-pathological examination.<sup>1</sup>

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### Sudden Cardiac Death, Anomalous Origin of Left Coronary Artery, Cardiopathology

### H23 Things Are Never What They Seem—A Fatal Injection in a Drug Abuser: Hemorrhagic Shock, Pulmonary Embolism, Fulminant Sepsis, Acute Methadone Poisoning, or Medical Liability? A Case Report

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of integrating the circumstantial, clinical, toxicological, and histopathological data in the definition of the cause of death in a drug-related death with contextual copious hemorrhage.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the circumstantial data is not always sufficient to define the exact causal seriation of the death; sometimes it may induce the medical examiner to focus attention on facts and elements secondary to the definition of the cause of death.

The identification of the cause of death derives from the application of a rigorous forensic methodology. In the case submitted, the historical-circumstantial data was indicative of a hemorrhagic shock from probable injury to the brachial artery. The toxicological investigation, however, showed high levels of methadone such as to independently justify the death.

This study presents the case of a 44-year-old man with a history of drug addiction being treated with methadone, found unconscious by his mother on the floor surrounded by a large blood patch. The man was rescued and transported to a hospital where he died a few hours later. The body was subjected to forensic examinations; a lesion of the brachial artery was found. The toxicological examination showed a high level of methadone in the blood (935ng/ml, reference: <10ng/ml).

Methadone is a drug used in heroin detoxification programs. The initial dose in medication-assisted methadone treatment corresponds to 20–30mg once daily and should not exceed 30mg. The maintenance dose range is 80–120mg per day, with dosing adjustments being made over the first week based on withdrawal symptoms.<sup>1</sup> Accumulation of methadone can lead to sedation, respiratory depression, respiratory arrest, and death.<sup>2</sup> Respiratory depressive effects can also occur at doses below 30mg in non-tolerant subjects.<sup>3-5</sup>

The death of the subject, according to the autopsy investigations and pathological findings, must relate to respiratory pneumonia of central origin, resulting from the depressive-inhibitory toxic action of the breathing centers due to the action of methadone in the subject with simultaneous blood loss.

The exact identification of the cause and means of death in each case of drug-related death is articulated through a methodological path that considers the circumstantial data, the results of the sector experiment, as well as the laboratory tests, according to what is advocated by the most careful doctrine coroner who recalls how the “diagnosis in terms of acute or chronic toxicity intoxication is a paradigmatic example of the need to correlate anamnestic-clinical data, anatomical-pathological, macro and microscopic findings, and chemical-toxicological findings.”<sup>6</sup>

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#### Drug Abuse, Hemorrhagic Shock, Methadone

## H24 Sudden and Unexpected Death After an Induced Abortion: Medical Responsibility or a Hidden Cause?

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of occult pheochromocytoma as a cause of sudden and unexpected death in young patients.

**Impact Statement:** This presentation will impact the forensic science community by presenting the importance of the medicolegal investigation in a case of sudden and unexpected death immediately after a medical procedure.

Pheochromocytoma (PHEO or PCC) is a rare catecholamine-producing tumor originating in the adrenal medulla.<sup>1</sup> Symptoms are due to catecholamines over-production and related to sympathetic nervous system hyperactivity (i.e., hypertension, tachycardia, sweating, headache, pallor) or a mass effect (i.e., abdominal pain or bowel obstruction). Acute crises can be spontaneous or may be triggered by medications, tumor manipulation, or anesthesiology procedures.<sup>2</sup>

This study reports the case of a 36-year-old woman admitted to the gynecology department to undergo an abortion at seven weeks of gestational age. Obstetric history was positive for three term pregnancies (two normal spontaneous vaginal deliveries and one caesarean delivery). The patient did not report any illness or medication use. At the preoperative anesthesiology evaluation, vital signs were unremarkable. A medical abortion was performed, followed by Dilation and Curettage (D&C) under epidural anesthesia. The D&C lasted five minutes and immediately after the procedure, the patient developed a severe hypertensive crisis with cardiogenic shock. The patient required intensive resuscitation procedures, including inotropic agents and extracorporeal membrane oxygenation. She subsequently developed hepatic failure that led to coagulopathy. A computed tomography scan revealed a previously unknown left perirenal mass. Eventually, the patient died due to multiorgan failure.

An autopsy was ordered to clarify the cause of death and to assess any medical responsibility. At the internal examination, the left mass was found to originate from the adrenal gland; upon gross examination, the cut surface of the mass was diffusely hemorrhagic. Histopathology of samples from the adrenal mass using hematoxylin-eosin stain was suggestive for PHEO.<sup>3</sup> The immunohistochemistry analysis on the same samples demonstrated strong and diffuse positivity for chromogranin A in all fields. In addition, a medical inquiry with the woman's relatives revealed that the deceased was affected with hypertension treated with triple-combination therapy. In the light of these findings, death was ascribed to an acute hypertensive crisis caused by an occult adrenal PHEO. PHEO has been referred to as the great mimic because the symptoms are quite common and sometimes it can be suspected only after paroxysmal episodes such as hypertensive crises. Unmanaged and/or undiagnosed PHEO is dangerous and can lead to serious and potentially lethal cardiovascular complications due to the acute massive secretion of catecholamines.<sup>4</sup> In this case, the patient was affected by an undiagnosed PHEO that triggered a lethal hypertensive crisis during a minimally invasive gynecological procedure under local anesthesia. Medical malpractice was excluded because the tumor was occult and the patient did not report her complete medical history. In conclusion, an unknown PHEO can lead to sudden and unexpected death in young patients. In some instances, the diagnosis of PHEO can only be formulated after a complete medicolegal investigation.

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### Sudden and Unexpected Death, Pheochromocytoma, Abortion

## H25 The Walking Dead: A Strange Case of Falls From a Staircase

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the role of postmortem Computed Tomography (CT) images in the quick interpretation of blunt traumatic injuries, especially when circumstantial data is not available.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that a combined classical and new forensic method with the use of Bloodstain Pattern Analysis (BPA) and postmortem CT is important to explain possible falling patterns and manner of death in individuals presenting lesions from falls down staircases without contextual information.

This work presents the case of a 64-year-old female found dead at home. The body was found prone on the middle of the second flight of a double U-shaped staircase with one square landing. At the crime scene, the primary blood pool was found at the bottom of first flight and a swipe pattern of blood was found on the steps of second flight coupled with a small amount of pool blood around the head of victim. The external examination showed extensive laceration and abrasion of the left side of the face (nose, eyebrow, eyelid, cheek) and multiple bruising on the back of the hands and arms. Postmortem CT was performed immediately and showed comminuted fractures of the nasal pyramid, bilateral maxillary sinus, left medial orbital wall, left cheekbone, fractures and dislocation of the middle-lower cervical spine (C4, C6 vertebrae) with fractures of spinal processes and severe deformity of the spinal cord. The autopsy confirmed fractures and revealed perivertebral spine hemorrhage coupled with contusion hemorrhages and transection of the C4 spinal cord. Microscopical examination confirmed the vitality of lesions and revealed massive pulmonary edema and myocardial contraction band necrosis, suggesting an acute condition of spinal shock as the mechanism of death. Toxicological analysis was negative.

Falls down stairs frequently occur within domestic environments. The skeletal blunt force trauma resulting from fatal falls involving stairs is complex. Frequently, it is not possible to understand the cause of the fall and the manner of death just with an external examination. There are multiple ways an individual may fall when stairs are involved.<sup>1</sup> Thus, the importance of a careful combination of the accurate analysis of evidence collected from crime scene and the evidence collected from the forensic pathologist is of the utmost importance.

From the result of the Crime Scene Investigation (CSI), it was apparent that the victim fell onto her face and struck the left side of her face, not on the step where found, but on a flat surface such as the bottom of first flight where the bigger blood pool was found. The postmortem CT quickly suggested that the victim had sustained a severe spinal cord lesion and wasn't able to move from the primary site of impact. Contrary to expectations, the bloodstain pattern analysis showed drag marks called a "swipe pattern" on the steps of second flight, a bloodstain pattern with characteristics that indicated relative motion between the two surfaces. So it was possible to suspect that the victim was dragged downstairs from the primary blood pool found at the bottom of first flight. On the basis of CSI and postmortem CT, it was immediately possible to exclude an accidental fall. The CSI and autopsy findings helped the forensic pathologist in reconstructing the sequence of events. In all probability, the victim was pushed down from a first flight with an impact onto her face against the square landing where the blood pool was found, causing an acute hyperextension injury of the cervical spine, with resultant profound spinal shock and instantaneous death. Later, the suspect tried to push the victim down the second flight of stairs. A suspect was arrested. He admitted that he was guilty.

This case underlines the usefulness of a combined forensic approach to determine the manner in which the death occurred in a case of blunt trauma. In addition to the classical forensic method, the use of BPA and postmortem CT is also important to determine the possible falling patterns from stairs.

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### Falls, Staircase, Blunt Trauma

## H26 Suicides by Barbiturate Intoxication

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**Learning Overview:** After attending this presentation, attendees will be more familiar with the toxic and lethal effects of barbiturates and the increased incidence of suicides resulting from their use.

**Impact Statement:** This presentation will impact the forensic community by highlighting the use of barbiturate medications in suicides and examine how the internet and publications are influencing this suicide method.

**Background:** Barbiturates are a class of sedative/hypnotic drugs used mainly as antiepileptics and for induction of general anesthesia. Some states use them in physician-assisted suicides/euthanasia and for capital punishment via lethal injection. Because of the high risk of overdose and no available antidote to reverse toxicity, their use in clinical practice has been largely replaced by benzodiazepines.<sup>1</sup> Now they are primarily used to anesthetize animals during surgery and euthanize them in veterinary practice.<sup>2-4</sup> Popular media, books, and information on the internet boasts barbiturates provide a peaceful method of suicide, which has led to increased interest in obtaining them from areas where they are less regulated.<sup>2,3,5,6</sup>

**Case 1:** A 55-year-old male (a veterinarian) made a 911 call that was traced back to his business address where he was found unresponsive with an intravenous catheter in the right antecubital fossa attached to two bags labeled "Veterinary 0.9% Sodium Chloride Injection" and "Veterinary Lactated Ringer Injection." On the counter near the decedent was a bottle of veterinary euthanasia medication containing pentobarbital sodium and phenytoin sodium. Toxicologic analysis of the blood revealed ethanol, pentobarbital, and phenytoin.

**Case 2:** A 63-year-old male was found deceased in his residence after law enforcement had received an email from the subject stating there would be a deceased body at his residence. Scene investigation revealed personal documents, books, and letters with Post-it® notes attached expressing who should receive which document. A bottle of sodium pentobarbital was also located on scene with instructions of how to administer it. A plastic cup containing a crushed white substance was located near the subject. Toxicological analysis of the blood revealed pentobarbital and diphenhydramine.

**Case 3:** A 68-year-old male with a history of chronic obstructive pulmonary disease left his residence after an argument with his wife. When he returned, he reported that he had taken "end of life medication" that he had purchased in Mexico (~30 pills of phenobarbital) with intentions of ending his life. Toxicological analysis of his blood revealed cannabinoids, fluoxetine, norfluoxetine, phenobarbital, bupropion, hydroxybupropion, and ethanol.

**Conclusion:** Although barbiturates are not used as commonly in clinical practice, they are still used to treat certain conditions and can be obtained from internet orders as well as in veterinary practices.<sup>1,2,5,7,8</sup> Pentobarbital is a very addictive drug categorized as a Drug Enforcement Administration (DEA) Schedule II substance.<sup>7</sup> Toxic levels of pentobarbital in the blood range from 8–24mg/L and lethal levels range from 15–241mg/L. Phenobarbital has less addictive potential and is categorized as a DEA Schedule IV substance with toxic levels in the blood ranging from 35–253mg/L and lethal levels ranging from 48–348mg/L.<sup>9</sup> Toxic/lethal effects of these drugs include airway compromise, cardiovascular collapse, coma, and death.<sup>1</sup> A study was performed on 51 cases in which barbiturates were detected in postmortem blood samples; of these 51 cases, 17 were ruled as suicides in which barbiturate toxicity was the primary cause of death. Within these 17 cases, 14 cases were due to pentobarbital toxicity and 3 were due to phenobarbital toxicity.<sup>10</sup> Although barbiturates are still not commonly used in suicides, with the increasing number of online drug sellers and the accessibility of suicide kits, the number of suicide deaths using barbiturates is on the rise.

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### Phenobarbital, Pentobarbital, Suicide

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## **H27 An Extremely Rare Case of Hemorrhagic Shock Caused by an Early Post-Implant Saphenous Vein Graft Pseudoaneurysm Rupture**

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**Learning Overview:** After attending this presentation, attendees will have discovered a rare complication of Saphenous Vein Graft (SVG) implantation. In this case, it presented with hemorrhagic shock instead of cardiac tamponade, as would have been expected. SVG rupture may lead to patient death and medical liability claims.

**Impact Statement:** This presentation will highly impact the forensic sciences community because it demonstrates that surgeons and pathologists have to explore the possibility of an SVG rupture in cases of post-surgical hemorrhagic shock, even if it is an extraordinarily unusual event.

**Introduction:** The SVG is a natural blood vessel used for surgical revascularization of a coronary. This technique is an authentic life-saving tool, and its use is still widespread today.<sup>1</sup> The procedure involves early and late complications. Late complications include restenosis or occlusion of venous bypass, progressive atherosclerosis of native coronary arteries, graft's aneurysms, intracoronary steal syndrome, coronary arteriovenous fistula, and aortic dissection at the anastomotic site.<sup>2</sup> Aneurysmal dilatation and pseudoaneurysm degeneration of SVGs have been rarely described in scientific literature.<sup>3,4</sup> Their rupture occurs in only 8% of cases with catastrophic complications.<sup>5</sup> In this presentation, a rare case of fatal hemorrhagic anemia after SVG pseudoaneurysm rupture is presented.

**Case Report:** A 53-year-old man was recovering after a syncopal episode. At the hospital, physicians diagnosed severe aortic stenosis, which required surgical treatment for valve replacement. During the procedure, the patient suffered a sudden episode of Ventricular Fibrillation (VF). The physicians hypothesized that myocardial hypoperfusion was caused by ostial occlusion that occurred during valve implantation. For this reason, the surgeons performed an immediate double bypass through a bilateral autologous SVG. After only four hours, the hemoglobin level dropped inexorably. An echocardiography performed seven hours after the surgery showed a small amount of pericardial effusion. The day after, the patient's clinical condition became critical; no blood transfusion or epinephrine injections could prevent hemorrhagic shock and death.

The Judicial Authority investigated if medical malpractice might be involved and ordered an autopsy. The main issue, in this case, was to understand whether the doctors could have prevented the patient's death.

The autopsy revealed rupture of a pseudoaneurysm in the left coronary graft, which caused massive blood leakage. The pericardium appeared vastly infiltrated and full of clots. Pleural cavities contained massive quantities of blood, respectively 830ccs on the right and 230ccs on the left. Histological examination showed that the fissure of the SVG occurred first in the deepest layer and spread to the more superficial ones. The absence of clinical signs of cardiac tamponade, like it could be logically expected, and the impossibility to instrumentally investigate blood vessels, made clinical diagnosis extremely challenging.

Autopsy investigation allowed assessment of the cause of death: hemorrhagic shock caused by the SVG's pseudoaneurysm rupture. A silent, invisible, and unpredictable time bomb implanted in the patient's chest developed between the explant and re-implantation of the graft. Therefore, necropsy examination was essential to relieve the medical staff and demonstrate that pseudoaneurysm rupture can be an insidious early complication in bypass surgery.

In this report, a rare case of hemorrhagic shock due to SVG rupture soon after the implant is presented. Thanks to the forensic investigation, it has been demonstrated that the pseudoaneurysm rupture complication can occur even a few hours after the surgery and manifest with unusual characteristics. So, this event, even though atypical, should be suspected and investigated in good medical practice. In the same way, pathologists have to consider this event as a possible diagnosis of death in cases of hemorrhagic shock.

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### **Venous Grafts, Pseudoaneurysm Rupture, Hemorrhagic Anemia**

### H28 “She Don’t Lie, She Don’t Lie, She Don’t Lie—Cocaine!” Homicide, Suicide, or Accident? The Unresolved Question About Drug-Related Deaths

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**Learning Overview:** After this presentation, attendees will be able to recognize the symptoms and signs of overdose from cocaine ingestion.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from ingestion of a large quantity of cocaine.

A 2017 general population survey on drugs found that a third of the Italian population aged 15–64 years had used a psychoactive substance at least once in their lifetime, and one in ten had done so in the previous year. The majority of consumers are male, although the incidence increased in both sexes. For what concerns cocaine, it is a drug whose use varies from occasional to compulsive. Repeated use of cocaine can lead to addiction and other serious harm. Cocaine can be taken in different ways: depending on the chemical-physical form of the substance, it can be inhaled, injected, smoked, or ingested. There is no safe way to take cocaine, since any modality can lead to the absorption of toxic doses, causing serious cardiovascular or cerebrovascular problems and, in some cases, sudden death. Cocaine ingestion, however, is the most unusual form of cocaine intake and it is mainly chosen for national and international transport. The absorption of ingested cocaine already begins in the oral mucosa and continues in that of the gastrointestinal tract. Through the oral route, the substance bioavailability is 30–40%. The maximum duration of effects of an average dose (20–50mg) ranges from 45 to 90 minutes. Several studies have shown the potentially lethal consequences of cocaine packet ingestion.<sup>1-5</sup>

A 36-year-old Italian man who had suffered from a depressive disorder subsequent to the recent death of his mother was found lifeless on the sofa of his home. The evening before the discovery, after having had dinner and simultaneously drinking a small amount of wine with his father, he had a psychotic crisis with pantoclastic manifestations. Several psychoactive drugs, such as lithium and clomipramine, were found at the crime scene.

At the external examination, a cadaveric rectal temperature of 41.3°C was registered, compared to an ambient temperature of 31.3°C. Moreover, several skin abrasive injuries were identified on the upper and lower limbs, as accidental consequences of the premortem psychotic crisis. During autopsy examination, a circular plastic film was found in the hypopharynx. A series of six other similar plastic films were then identified in the stomach. Toxicological investigations revealed the presence of cocaine equal to 466ng/L (cut-off 10ng/mL), metabolites of cocaine all above the cut-off, and alcohol equal to 0.26g/L in the cadaveric blood. No psychoactive drug (or their metabolites) was found.

Usually, the typical mode of assumption of cocaine is inhalation. The case here presented represents the first documented one in Italy of a fatal overdose resulting from the ingestion of large quantities of cocaine, the toxicity of which has been amplified by the presence of alcohol. The intake of the substance still packaged in its envelopes does not allow a differential diagnosis to be made between the hypothesis of suicide or accidental death that could have been occurred after a failed attempt at concealed drug transport.

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#### Cocaine Ingestion, Toxicology Findings, Psychotic Crisis

### H29 Another Case of Femicide? No, That's Another Case of Underlying Sudden Unexpected Death in Epilepsy (SUDEP)

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**Learning Overview:** After attending this presentation, attendees will understand that SUDEP is still an underestimated and difficult to diagnose cause of death and that it is very important protecting epilepsy through an evaluation of the environmental conditions and periodic medical strictly controls.

**Impact Statement:** This presentation will impact the forensic science community by showing the crucial importance of a full medicolegal investigation (i.e., clinical history collection, autopsy, and histological and toxicological studies). Furthermore, there is a need to educate officials at all levels about this diagnosis in persons who have epilepsy with no other cause of death.

SUDEP is defined as “the sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy with or without evidence of a seizure, and excluding documented status epilepticus, in which postmortem examination does not reveal a structural or toxicological cause for death.”<sup>1</sup> The most common pathologic finding in autopsies is pulmonary congestion/edema (~50%); there is no evidence of neurodevelopmental abnormalities, which are often the etiology of epilepsy. While the mechanisms of death in SUDEP are unknown, these discoveries suggest that cardiogenic mechanisms may be involved in the sudden death of these patients. Cardiac and pulmonary pathological abnormalities are frequent among SUDEP cases, most commonly pulmonary edema/congestion and focal interstitial myocardial fibrosis. There are frequent reports of persons dying alone in their sleep. It is assumed that death occurs following a seizure, but since the deaths are often unwitnessed, this is only an assumption. The most important risk factor appears to be poor seizure control.<sup>2,3</sup>

The present case concerns a 43-year-old Italian woman found dead in her bed, in the prone position, with her face pressed against the pillow. The medical history suggested epileptic disease from infancy under pharmacological treatment, with persistence of episodic seizures, the last of which happened the night before; no other pathology was documented. During the police investigation, the house was found in order. There was also a violent relationship with her husband, who was addicted to alcohol and drugs. An immediate rough examination of the body showed small bruises on the neck, but the case was closed for natural death, without further investigation. A forensic autopsy was performed one month after her death, after the parent's claims to the police of suspicion of homicide committed by the husband of the victim. The corpse was in putrefaction; the external examination showed the signs of old bruises to the arms and neck. The internal examination mainly revealed an absence of injuries and or hemorrhagic infiltrations, while pulmonary edema was found. The brain has not been studied due to advanced autolysis. The heart showed a normal size with early coronary artery atherosclerosis. The evaluation of histological specimens showed cardiac interstitial fibrosis, massive edema of the lungs associated with multi-organ congestion, liver steatosis, and no vital signs in the epidermal lesions. Toxicological analysis were negative.

This case underlines the need to keep attention levels high in the forensic analysis of all so-called “seizure-related deaths” to exclude with certainty the initial suspicion of homicide and to make a final diagnosis of SUDEP.

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#### SUDEP, Forensic Investigation, Differential Diagnosis



### H30 The “Pathologic” Mother-Son Bond: An Unusual Case of Matricide With “Overkill”

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**Learning Overview:** After attending this presentation, attendees will understand that matricide is a rare and aberrant event in which the reasons for the murder could be found in the complex and pathological bond between mother and son/daughter.

**Impact Statement:** This presentation will impact the forensic science community by highlighting a rare case of matricide with “overkill” in which the perpetrators, after killing the mother, plug needles in the face of the cadaver to simulate the punishment his mother inflicted on him when he was a child.

Matricide is among the rarest murder and has always been considered one of the most abhorrent crimes. Studies on matricide by a son highlighted that most perpetrators are single adults with an intense relationship with their mother, a lack of interest in other women, a feeling of social inferiority, and an absent or passive father.<sup>1</sup> These subjects often reported feeling that their mothers were either ambivalent toward them or excessively domineering.

Matricide is classically committed in the victim’s home, and a weapon is usually used, although asphyxia is also common. Moreover, matricide is described as associated with “overkill” in some cases.<sup>2</sup>

A 73-year-old woman was found dead in her home by police. The police intervened because they were alerted by a neighbor who had heard the woman scream and ask for help. In the house, the young adopted son attacked the agents with a knife; he was then disarmed and arrested. The forensic pathologist performed the crime-scene investigation, which observed a living room mess and the woman’s body lying on the floor covered with furnishings. The preliminary external examination revealed skull and facial trauma and the presence of needles strung in various regions of the face. At the autopsy, the needles were found, respectively: one in the left eye, five in the left zygomatic region, and one in the left mandibular region. Several maxillo-facial fractures and brain hemorrhage were found; moreover, bruises, contusions, excoriations, and lacerations were observed on the body, also at the neck. There were multiple bilateral rib fractures and hemopericardium due to right ventricle laceration.

Based on the forensic investigations (crime scene analysis, circumstantial data, autopsy, and blood pattern analysis), the murder was reconstructed, supporting the extreme violence through which the young men attacked the mother, causing her death from severe trauma to the head and chest.

Even if the literature reported that most matricides suffer from psychotic disorders, especially schizophrenia, this is a rare case in which non-psychiatric illnesses were diagnosed. A “pathological” mother-son bond was described, and the murder reason was brought back to the vexatious dynamics perpetrated by the mother since the son was a child; the mother punished the boy with needles strung in the face. The reconstruction of the mother-son relationship, as well as the life experiences of both, allowed consideration that the murder was a dramatic attempt to “break” the destructive bond among them.

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#### Matricide, Overkill, Mother-Son Bond

## H31 Charred Bodies: Homicidal, Suicidal, or Accidental Death? A Case Series

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**Learning Overview:** After attending this presentation, attendees will have acquired a better insight on the importance of a multidisciplinary approach for the determination of cause and manner of death—homicidal, suicidal, or accidental—when charred bodies are recovered

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of a proper interpretation of the results obtained from different forensic investigations carried out on severely charred bodies in order to correctly determine manner and cause of death, as well as its dynamics when the obtained data are combined with the information provided by specific professional figures in selected cases.

Charring is a process responsible for a wide range of heat-related injuries, which often produce deep destructive transformations of organs and tissues following a prolonged exposure of the body—or parts of it—to temperatures  $\geq 300^{\circ}\text{C}$  as a consequence of a combustion process between a fuel and an oxidizer (usually the oxygen in the surrounding atmosphere). Depending on the body parts involved and the temperatures and time of exposure, the destructive effect on human remains can largely vary from localized burns/charred areas to complete incineration. As such, depending on both the fire entity and the extent of the charred body surface, issues may arise concerning: (1) identification, especially when no clothes or personal objects are recovered on cadavers and physiognomic features appear severely impaired; (2) primary cause of death, that is to say whether the fire effects occur before or after death; and (3) manner of death, whose elucidation is of utmost importance in cases of homicide since charring represents a frequent attempt to destroy forensic evidence and prevent the cadaver recovery.

Under the heat effect in cases of extensive charring, skin appears dry and blackish, with burnt or almost absent hair; dehydration is responsible for tissue retraction followed by an overall reduction of the body surface, together with muscle contracture; the consequential extreme flexion of upper and lower limbs lets the cadaver assume a typical “pugilistic” pose. Bones usually undergo the so called “calcination” phenomenon, which makes them more frail, and thus more prone to non-vital fractures and/or amputations; epidural heat hematomas can also be detected as consequence of heat-induced suction due to the volume reduction of the brain. Since these findings, although characteristic, taken alone are almost unspecific, an aid in understanding the manner and dynamics of death must be provided by additional investigations.<sup>1-3</sup>

To this aim, cause and manner of death have been investigated by a multidisciplinary approach in 21 cases of severely charred bodies. Along with circumstantial data, information on mental health status and autopsy, and whenever possible, additional findings have been provided by: histology, carried out on 14/21 cases, which gave rise to unspecific findings; Computed Tomography (CT) scan, carried out on 17/21 cases in order to detect and eventually characterize internal injuries not related to fire and/or foreign bodies; and toxicological investigations, carried out on 17/21 cases in order to detect either the presence of abuse substances and the levels of blood carboxy-hemoglobin as an indicator of aspiration occurrence before death. A novel investigation, Scansion Electron Microscopy with Energy-Dispersive X-ray (SEM/EDX), was carried out only in case number 3 for the evaluation of both heat-induced bone alterations and metallic residues as indicators of the cadaver recovery site. In 7/21 cases, they rely on an engineer expertise, also revealed fundamental for the comprehension of the dynamics of the event.

The combined interpretation of the different findings thus allowed the statement of 11 cases as accidental deaths, 6 cases as homicidal, and 4 cases as suicidal.

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### Charred Bodies, Forensic Investigations, Manner of Death

### H32 The Immunofluorescence Survey and the Persistence of SARS-CoV-2 After Death

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of the use of immunofluorescence as a rapid test to detect the persistence of SARS-CoV-2 infection in cadavers.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of the persistence of SARS-CoV-2 18 days after death in a buried corpse.

To date, over 4.5 million deaths due to SARS-CoV-2 infection have been recorded worldwide.<sup>1</sup> Contagion occurs mainly through the release of droplets, during speech and respiration, and therefore the contact of viral particles with the epithelial surfaces, in particular on the bronchi and conjunctiva.<sup>2</sup> The analysis of nasal and oro-pharyngeal swabs for the search for viral RNA is the primary tool for detecting the presence of infection through the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method, which is based on the amplification of the traits of genetic material of interest. Its use, even in the forensic field, is recommended by scientific societies and by Italian guidelines.<sup>3</sup> The search for the proteins (antigens) of the virus by immunofluorescence is, however, able to prove in cases of positivity, a greater integrity of the viral particles. This is an essential prerequisite to be able to hypothesize the survival of virions.

This study brings knowledge to the international scientific community of the case of the finding of a double positivity to nasopharyngeal swabs, carried out during an autopsy, on the corpse of a man who died 18 days earlier, analyzed with both methods. The man died from the development of massive interstitial pneumonia (also documented on Computed Tomography [CT] images) associated with COVID-19 pulmonary vascular microthrombosis. The peculiarity of the case is that, following the death, the body was never kept in a cold room, but it was buried. The corpse was placed in a body bag inserted into a galvanized wooden crate, in turn contained in a metal casing, and was subsequently exhumed to allow the autopsy. This made it possible to detect the persistence of the viral genetic material (RNA) found on RT-PCR, as well as, above all, of the viral antigens, detected thanks to immunofluorescence, 18 days after death. The assay used is a lateral flow immunofluorescence sandwich assay for the qualitative detection of the SARS-CoV-2 nucleocapsid protein antigen.

This research intends to emphasize the importance of performing nasopharyngeal swabs to be analyzed by immunofluorescence on cadavers with suspected or confirmed COVID-19 infection. This test has, in fact, the advantage of giving a quick answer and, in case of positivity, of identifying the bodies at greater infectious risk. Furthermore, it allows us to formulate hypotheses about the survival time of the virus—an indispensable prerequisite for contagion—in the corpses of infected people at the time of death and stored in ambient temperature conditions.

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#### Forensic Science, Postmortem Swab, Immunofluorescence

### **H33 Hepatodiaphragmatic Interposition of the Colon: Radiological Evidence and Autopsy Findings in a Case of Chilaiditi Syndrome**

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**Learning Overview:** After attending this presentation, attendees will better appreciate the characteristics of a rare anatomical condition identified as Chilaiditi syndrome through the description of a case of sudden death that occurred after admission to the emergency department.

**Impact Statement:** This presentation will impact the forensic community through the description of autopsy findings related to a condition of prevalent radiological finding.

Chilaiditi syndrome is a rare condition, caused by an interposition of the colon or small intestine between the liver and diaphragm. The anatomical condition is associated with clinical manifestations; the most frequent presentation is abdominal pain, anorexia, nausea, vomiting, flatulence, constipation, abdominal tension followed by respiratory distress, and, less frequently, cardiac symptoms such as chest pain and arrhythmias. These manifestations are more severe when the subject assumes a supine position, and, only rarely, involve several organs at the same time. Gastrointestinal involvement can be of varying degrees of severity up to the acute abdomen. On the contrary, the anatomical finding in the absence of correlated symptomatology only allows highlighting the presence of the Chilaiditi sign in the absence of a syndromic context.

The present report concerns the death of a 59-year-old man that occurred suddenly a few hours after being admitted to the emergency department for abdominal pain, constipation, sweating, skin pallor, and abdominal distension. During hospitalization, the clinical conditions were substantially stable with normal vital signs; the only relevant evidence was provided by an abdominal Computed Tomography (CT) scan which documented the presence of the previously never-objectified Chilaiditi sign. Despite this, the death was unexpectedly ascertained after a few hours; for this reason, an autopsy was required to establish the cause of death.

The autopsy assessment confirmed the hepatodiaphragmatic interposition of the colon associated with diaphragmatic lifting and visceral adhesions. In addition, an increase in heart volume and weight related to the subject's gender, age, height, and weight, associated with a condition of systemic atherosclerosis, was found. No findings compatible with intestinal occlusive pathology were highlighted. In consideration of the clinical history and autopsy evidence, organ and tissue samples were collected for subsequent histological investigations.

The histological examination revealed the presence of coronary artery disease and widespread interstitial fibrous substitution phenomena in the heart. A modest amount of alveolar edema was also evident in the lungs. The colon wall showed no noteworthy structural changes.

At the end of the investigations, the cause of death was identified as acute heart failure in the context of dilated heart disease associated with coronary atherosclerosis and myocardial interstitial fibrosis.

In conclusion, this presentation offers an extensive anatomical detail of a condition that presents mainly, if not solely radiological, description in the literature. In addition, some autopsy procedural insights are provided for the diagnosis of pathological conditions possibly associated with Chilaiditi syndrome.

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#### **Chilaiditi Syndrome, Radiological Evidence, Autopsy Findings**

## H34 Rare Congenital Diseases and Infant Deaths: Postmortem Investigations of a Case of Fraser Syndrome

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**Learning Overview:** After attending this presentation, attendees will better appreciate the pathological implications of a rare congenital disease associated with high neonatal mortality: Fraser Syndrome.

**Impact Statement:** This presentation will impact the forensic community by sharing a multidisciplinary operative methodology that can be adopted in suspicion of genetic diseases involving multiple districts.

Fraser Syndrome is a rare autosomal recessive genetic disorder with an estimated prevalence (Europe) of 1/500,000 births. It is caused by mutations in FRAS1, FREM2, and GRIP1 genes and coding for extracellular matrix proteins also involved in the embryological development, whose mutation is believed to be responsible for a failure in apoptosis. The alterations can affect eye, urinary tract, reproductive organs, craniofacial structures, respiratory systems, heart, digestive tract, and skull, although the most characteristic manifestations of the pathology are cryptophthalmos and syndactyly. In the most severe cases, Fraser Syndrome has a high rate of prenatal or perinatal death, generally due to alterations in the upper aerodigestive tract and the excretory system; otherwise, extended or normal life expectancy have been reported.

The present report concerns the investigations conducted in a case of death in a 6-month-old child affected with Fraser Syndrome.

The analysis of the available clinical documentation revealed that the baby was born premature at the 34<sup>th</sup> week, due to the presence of ascites, polyhydramnios, anemia, and scarce fetal movements that required caesarean emergency section, following which he underwent invasive ventilation for the presence of respiratory failure. Due to the finding of multiple malformations, physicians suspected Fraser Syndrome. At the sixth month of life, the baby was found unconscious and not responding by the mother and was transported to the nearest emergency room. Despite the timely intervention of the doctors and the resuscitation maneuvers performed for about two-and-one-half hours, death was ascertained.

Postmortem investigations were carried out in order to define the causes of death and specify the conditions suffered by the child with preventive purposes. Prior to invasive investigations, the body underwent both Postmortem Computed Tomography (PMCT) and Postmortem Magnetic Resonance (PMMR).

Multiple pathological findings were observed during external examination, including dysmorphism of the head due to a net increase in posterior diameters, hypertrichosis, bilateral cryptophthalmos with microphthalmia of right eye, ogival palate, single transverse palmar crease at the right hand and two to three toes syndactyly bilaterally. Autopsy was performed according to Letulle technique; given the presence of abundant yellowish milk-like material in the upper airways and the observation of suggestive findings at radiological investigations; trachea and bronchi opening was performed at the autopsy table and revealed the presence of similar material in correspondence of the right main bronchus and the macroscopically explorable branches. The brain and the whole Letulle block were fixed in 10% buffered formalin, while samples of organs' parenchyma for genetic investigations were taken and stored in the freezer at -20°C. The diagnosis of death from bronchoaspiration of food material was confirmed both by the revision of the fixed organs and by histopathological investigations, which revealed the presence of birefrangent material in the airways.

In conclusion, the clinical autopsy and the further investigations carried out made it possible, on the one hand, to obtain with certainty a diagnosis of death and, on the other, to document the typical alterations of a rarely described condition with the desirable repercussions not only for postmortem diagnostics, but also for the clinic on the living.

### Fraser Syndrome, Neonatal Deaths, Postmortem Investigation

### H35 The Role of Superstitions In Sexual Assault: A Case Discussion

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**Learning Overview:** After attending this presentation, attendees will be aware of how superstitions and blind beliefs make women vulnerable to sexual assault.

**Impact Statement:** This presentation aims to provide an insight into how superstitions, myths, and beliefs make women fall prey to people who then commit crimes, including sexual assaults.

Superstition refers to any belief or practice that is caused by supernatural causality and which contradicts modern science. The acceptance of superstitions and paranormal healing are claimed by fake shamans and believed by many. It is not only confined to the uneducated but also the educated and developed population.<sup>1</sup> There are various sociological factors—regional beliefs, local population structure, and social influences—as well as the belief in the powers of paranormal beings in solving problems of human beings. People believe that such practices are to be followed silently and not questioned or analyzed on scientific lines. The effect of superstition is seen more among women and in those who are emotionally vulnerable. The practitioners of such faiths even exploit childless women sexually under the pretext of curing their infertility. Lack of education, failures, and domestic problems make domination and sexual harassment added to the woes of women, forcing them into the trap of superstitions.

Counseling centers have also been started to dissuade women in distress to turn to superstitions for solace. It even had to take the death of an activist for the first Act to be passed in the Parliament.<sup>2</sup> However, education remains the best solution to this.

The present case discusses one such disturbing incident and the various measures that can be undertaken to combat such issues. This presentation also discusses the responsibility and accountability of the society and lawmakers to improve the mindset and imbue rationality and science in the mind of the people.

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**Superstition, Myths, Sexual Assault**

### H36 Standards Development Activities in Forensic Medicine

*Jason M. Wiersema, PhD, Harris County Institute of Forensic Science, Houston, TX; Keith Pinckard, MD, PhD; Travis County Medical Examiner's Office, Austin, TX*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to forensic medicine.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities within forensic medicine. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in forensic medicine will be discussed, including standards that have been added to the OSAC Registry, OSAC proposed standards, standards published by SDOs and other authoritative bodies that have yet to go through the Registry approval process, and documents currently in development by the Subcommittees of the Forensic Medicine SAC. The subcommittee priorities for upcoming work products and other initiatives will be presented, including opportunities for supplemental training related to discipline-specific standards, and additional resources designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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#### Forensic Medicine, Standards, OSAC

## H37 Standards Development Activities in Wildlife Forensic Biology

*Kimberly Frazier, MS\*, Wyoming Game and Fish Department, Wildlife Forensic and Fish Health Laboratory, Laramie, WY*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of wildlife forensic biology.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities in wildlife forensic biology.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in wildlife forensic biology will be discussed. These include:

- Recent standards that have been added to the OSAC Registry:
- American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 019, *Wildlife Forensics General Standards*, First Edition, 2019
- ANSI/ASB Standard 029, *Report Writing in Wildlife Forensics: Morphology and Genetics*, First Edition, 2019
- ANSI/ASB Standard 028, *Wildlife Forensics Morphology Standards*, First Edition, 2019
- ANSI/AASB Standard 047, *Wildlife Forensics Validation Standard—Validating New Primers for Sequencing*, First Edition, 2019
- OSAC proposed standards Wildlife Forensics Methods
- OSAC 2021-N-0001, *Wildlife Forensics Method—Collection of Known DNA Samples from Domestic Mammals*

Published standards from the ASB pending Registry approval process:

- ANSI/ASB Standard 046, *Wildlife Forensics Validation Standards—STR Analysis*, First Edition 2019
- ANSI/ASB Standard 048, *Wildlife Forensic DNA Standard Procedures*, First Edition, 2019
- ASB Standard 106, *Wildlife Forensics—Protein Serology Method for Taxonomic Identification*, First Edition, 2020
- ANSI/ASB Standard 111, *Standard for Training in Mitochondrial DNA (mtDNA) Analysis for Taxonomic Identification*, First Edition, 2020
- Documents currently in development at the OSAC or by the ASB:
- Genetic methods to determine an individual of potential hybrid origin
- Criteria for analyzing Short Tandem Repeat (STR) data in forensic wildlife cases when no allelic ladder is available
- Standard for the development and use of in-house sequence databases for taxonomic assignment of wildlife
- Method for geographic assignment of individual animals
- 2021-S-0006 Standard for the Use of GenBank for Taxonomic Assignment of Wildlife
- 2021-N-0001 Wildlife Forensics Method—Collection of Known DNA Samples from Domestic Mammals
- Wildlife Forensics—Standards for Construction of Multilocus Databases

Other highlights:

- Hosted an OSAC Wildlife Forensics Validation Discussion
- Developed several research needs

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### Wildlife Forensics, Standards, OSAC



### H38 Professionals of the Medicolegal Death Investigation Workforce—The State of the Talent Pipeline

*Jeri Ropero-Miller, PhD\*, RTI International, Research Triangle Park, NC; Hope Smiley-McDonald, PhD, RTI International, Research Triangle Park, NC; Crystal Daye MPA, RTI International, Research Triangle Park, NC; Kelly Keyes, BS, RTI International, Research Triangle Park, NC; Connor Brooks, MSc, Bureau of Justice Statistics, Dulles, VA*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of professionals in medicolegal death investigation and the needs associated with improving the workforce pipeline.

**Impact Statement:** This presentation will impact the forensic science community by presenting general staffing statistics, operations, training, and professional development data within the United States medicolegal death investigation system.

**Introduction:** In 2019, the United States Bureau of Justice Statistics (BJS) (2017-MU-CX-K052) administered the national Census of Medical Examiners and Coroners' Office (CMEC) to collect 2018 data describing the Medicolegal Death Investigation (MDI) in the United States. In 2017, 450,000 deaths were investigated by Medical Examiner and Coroner (ME/C) offices, accounting for approximately 20% of all deaths; more than 70,000 were overdose mortalities.<sup>1</sup> This was before the 2020 COVID-19 pandemic, which caused an additional 375,000 United States deaths.<sup>2</sup> The lack of highly skilled and trained MDI professionals, especially board-certified forensic pathologists, persistently and negatively affect the ability of ME/C offices to complete their investigations adequately, in a timely manner, and affordably. Publicly accessible BJS data were analyzed to report on the talent pipeline of autopsy pathologists, death investigators, autopsy technicians, toxicologists, and other professionals in this national census of more than 2,100 ME/C offices.

**Objective:** To analyze and discuss operational characteristics of ME/C offices in the United States to provide insight for staffing, workload, budget and operations, training, and professional development.

**Methods:** The 2018 CMEC was conducted during an 11-month period. A census questionnaire was designed and coordinated with forensic expert panel review and piloting of the instrument to select ME/C offices. As a mixed-mode data collection, ME/C responses were received by completed census questionnaires through mail, facsimile, computer-assisted telephone interviewing, and a web-based instrument. Questions and data providing details as to the operation of ME/C offices specific to staffing and the workforce were analyzed.

**Results:** This study provides insight and context for personnel (salary, certification), budgets, workloads, resources needed, and investigative practices within these ME/C offices. Where possible, the data are presented by type of office and size of jurisdiction, and selected comparisons with the 2004 CMEC administration will be made. The overall number of autopsies has increased since 2004, although the average caseload has gone down slightly. Although it has been estimated that there are about 400–500 forensic pathologists working in the United States, the 2018 CMEC found that this number may be higher.<sup>3</sup>

**Conclusion:** Attendees will gain an understanding of talent pipeline, current state of the ME/C workforce, training, and professional development practices within United States ME/C systems. Crucial statistics for the operation and function of our nation's death investigation system is discussed, fully realizing that the current state of staffing needs and workforce deficits is critical to public health and safety.

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2. Ahmad, F.B. et al. *MMWR Morb Mortal Wkly Rep* 2021;70:519–522.
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#### Medicolegal Death Investigation, Workforce and Talent Pipeline, Medical Examiner and Coroner Systems

## H39 Addressing the Future of Forensic Pathology in the Caribbean: The Need for Mentorship Among Caribbean Medical Students

*Ann-Marie Ming, BMedSc\*, Department of Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago; Catherine Morris MD, Department of Pathology, Port of Spain General Hospital, Port of Spain, Trinidad and Tobago; Alfredo Walker, MBBS, Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Toronto, Canada*

**Learning Overview:** After attending this presentation, attendees will be able to: (1) define and describe the role of mentorship in medicine in attracting interest in specialty training; (2) describe the state of forensic pathology in the Caribbean and how it can be improved through mentorship; and (3) describe a Caribbean medical student-led initiative that facilitates student mentorship by forensic pathologists.

**Impact Statement:** This presentation will impact the forensic science community by creating an awareness of the landscape of forensic pathology in the Caribbean as being a largely underrepresented and unrecognized field.

Over the past few decades, there has been an increase in autopsy cases with an inversely proportional forensic pathology workforce within the Caribbean region. Some Caribbean islands have no qualified forensic pathologists to perform medicolegal postmortem examinations, which results in these examinations being performed by the few anatomical pathologists in existence, inclusive of homicides and criminally suspicious deaths. This is not ideal and can lead to errors of interpretation and the potential for miscarriages of justice. The implications of this shortage run the gamut, leading to a backlog of cases, overworked forensic pathologists, delayed legal proceedings, freeing of the accused, distress to family members, an increased incidence of “inconclusive” findings, and overall poor quality of practice.<sup>1,2</sup>

Several factors account for this shortage inclusive of inadequate exposure to pathology and its subspecialties (such as forensic pathology) in the medical curriculum, misconceptions about the field, a lack of mentorship, the “introvert” personality stereotypes, and the imbalance between the reality of the fields versus the public perception as portrayed on television and in the media.<sup>3-6</sup>

Reform in the modern undergraduate medical curriculum has led to a focus on patient-centered approaches that have replaced the traditional subject-based curriculum. Limited pathology courses are therefore spread throughout the preclinical and paraclinical years, which facilitates minimal to no interaction with pathology and pathologists in clinical years and a lack of exposure to surgical pathology and the autopsy with a resultant reduced understanding of and disinterest in pathology.<sup>7,8</sup>

Within the University of the West Indies, exposure to forensic pathology is limited to observership of autopsies over four days during an anatomical pathology clerkship; with an average of 12 hours of exposure. A forensic pathologist is not guaranteed to be present and either an anatomical pathologist or house officer (post-internship junior doctor) provides instruction to medical students. These factors negatively impact proper exposure to forensic pathology and anatomical pathology and contribute to medical graduates considering careers in the more traditional specialties.

It is well known that the implementation of forensic pathology mentorship programs in medical schools will provide role models and career guidance to address these issues.<sup>4</sup> Additionally, involvement in a pathology interest group has been associated with medical students who chose a career in pathology.<sup>9</sup>

A survey conducted by the National Association of Medical Examiners' (NAME) Forensic Pathology Training Committee also found that the influence of a professor or mentor and exposure to the field in residency training were the most significant factors that influenced its members to pursue a career in forensic pathology.<sup>10</sup> Mentorship is therefore integral in influencing the choice of career. However, there is a lack of mentoring programs in medical schools in several countries, including throughout the Caribbean.<sup>11,12</sup>

**Conclusion:** The Pathology Club of the University of the West Indies was established in August 2020 together with an aligned mentorship initiative, the Caribbean Pathology and Laboratory Medicine Student Initiative (CPALMSI), supported by the Department of Pathology and Laboratory Medicine of the University of Ottawa, where there is a well-established and respected forensic pathology unit. This initiative has served to increase interest in pathology and provide mentorship and career guidance for Caribbean medical students.<sup>13</sup> Expansion of mentorship initiatives such as the CPALMSI is required to address the shortage of forensic pathologists in the long term.

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### Forensic Pathology, Caribbean, Mentorship

### H40 The Utilization of Blood Smears and Flow Cytometry to Diagnosis Hematologic Malignancies and Triage Cases for Autopsy

Jolee Suddock, DO\*, Office of the Medical Investigator, Albuquerque, NM; Molly Biggs, BS, University of New Mexico, Albuquerque, NM; Daniel Babu, MD, University of New Mexico, Albuquerque, NM; Clarissa Krinsky, MD, Office of the Medical Investigator, Albuquerque, NM

**Learning Overview:** After attending this presentation, attendees will have a better understanding of when to consider undiagnosed hematologic malignancies at autopsy, as well as how to collect blood and bone marrow, prepare smears, and utilize flow cytometry in the postmortem setting.

**Impact Statement:** This presentation will impact the forensic science community by proposing a protocol for the workup of suspected hematologic malignancies based on a case in which its use was successful. The aim is to improve diagnostic accuracy and aid in triaging autopsy cases.

Neoplasms presenting as unexpected death at autopsy are rare; however, hematologic malignancies have been reported as one of the most common causes.<sup>1</sup> Leukemic processes also often do not form discrete masses, unlike large mass-forming malignancies that can be more readily identified at autopsy or on postmortem imaging. Diagnosis relies upon the examiner's consideration at the time of autopsy. A number of case reports discuss the diagnosis of leukemia and lymphoma at autopsy; however, these diagnoses were primarily made by histologic examination of multiple organs or with the use of an extensive panel of immunohistochemical stains.<sup>2,3</sup> Flow cytometry has been utilized in forensic medicine in various ways; however, its use in the postmortem diagnosis of leukemia has not been well-established.<sup>4</sup>

In addition to diagnosis, utilization of blood smears and flow cytometry may aid in triaging autopsy cases. If the diagnosis can be accurately made using externally collected blood, a full autopsy may not be necessary. This is of particular benefit to offices that serve cultural and religious groups who request minimally invasive postmortem examinations.

This study presents a case of a 12-year-old girl who died at home. She had malaise, heavy menstruation, epistaxis, and fatigue in the days before her death. Externally she was markedly pale. Medical care was not pursued due to the religious beliefs of the family, and a minimally invasive examination was requested. This prompted the consideration of external examination with collection and evaluation of blood and bone marrow. Utilizing a large-bore needle, bone marrow was aspirated from the iliac crest and heart blood was collected. Smears were prepared on glass slides, allowed to air dry, and stained with Wright-Giemsa. Blood and marrow smears revealed numerous immature cells, prompting hematopathology consultation. Flow cytometry of collected blood identified blasts consistent with B-lymphoblastic leukemia. A full autopsy was completed after discussion with the family, and a lymph node was collected and was also found by flow cytometry to be involved by leukemia.

This case highlights that externally collected blood and bone marrow, with the use of flow cytometry, can aid in cause of death determination and may be used to triage select autopsies. Challenges to establishing protocol for the postmortem workup of hematologic malignancies include the viability of samples, the availability of staining kits and slides, and an association with a laboratory that performs flow cytometry. Future directions include establishing an optimal time frame for viability and evaluating the cost-effectiveness compared to traditional diagnostic methods.

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#### Leukemia, Autopsy, Triage

## H41 Histological and Immunofluorescent Imaging of Infant Bridging Veins

*Student Neil Harris, MSc\*, East Midlands Forensic Pathology Unit, University of Leicester, Leicester, Leicestershire, United Kingdom; Emma Cheshire, MS, East Midlands Forensic Pathology Unit, University of Leicester, Leicester, Leicestershire, United Kingdom; Guy Ratty, MD, East Midlands Forensic Pathology Unit, University of Leicester, Leicester, Leicestershire, United Kingdom; Roger Malcomson, PhD, Histopathology Department, Leicester Royal Infirmary, Leicester, Leicestershire, United Kingdom*

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**Learning Overview:** After attending this presentation, attendees will better understand bridging vein microanatomy.

**Impact Statement:** The diagnosis of Abusive Head Trauma (AHT) can be extremely challenging, with past controversies surrounding the mechanism of injury and the source of subdural bleeding. This presentation aims to provide novel micro-structural information on infant bridging veins from both head injured and non-head injured children to enable sample comparisons and to investigate potential markers of traumatic damage. Ultimately, this study hopes to provide a reliable and easily applicable diagnostic tool to forensic and pediatric pathologists, for the sensitive detection of damaged bridging veins in AHT cases.

**Background:** Damaged bridging veins are generally accepted by the majority of medical professionals as the source of Subdural Hemorrhage (SDH) in infants that have been subjected to AHT. However, damaged bridging veins can be difficult to visualize during postmortem examinations and on medical imaging such as magnetic resonance and computed tomography. There is also a paucity of information on the typical microanatomy of bridging veins, particularly in the infant population. When observed and photographed during postmortem examinations, some infant bridging veins are very small and extremely delicate.

Since 2013, the East Midlands Forensic Pathology Unit has developed and utilized a novel method for removing the infant calvarium using neurosurgical equipment. This technique leaves the underlying dura mater intact, enabling careful reflection of this membrane to visualize and dissect bridging veins. The Unit has also developed dissection methods using approximator clamps to extract bridging veins with minimized artifactual damage. These techniques have enabled the current microscopy study, detailing the typical microanatomy of infant bridging veins.

**Methods:** This study used histological, immunohistochemical, and immunofluorescent techniques to detail the typical microanatomy of infant bridging veins, retained with appropriate consent, during non-forensic postmortem examinations. Using specific markers (CD31 and smooth muscle actin), histological stains (Hematoxylin-Eosin [H&E], Masson's trichrome, and elastic Van Gieson's) and auto-fluorescent signals, this study documents the micro-structure of these vessels using light and fluorescent microscopy.

**Preliminary Results:** Preliminary results suggest that an infant bridging vein is composed of an endothelial lining, which is in close contact to a thin smooth muscle layer. Collagen bundles, surrounding the smooth muscle cells, are the main component of the bridging vein wall. Small amounts of elastin fibers are also noted within the bundles of collagen.

**Conclusion:** These insights into the typical structure of infant bridging veins will enable future comparisons between blood vessels retained from both AHT and non-AHT cases. It is hoped that these anatomical comparisons will enable the detection of trauma to infant bridging veins.

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**Bridging Veins, Head Injury, Pediatric**

### H42 Improving the Postmortem Examination Process Using Complete Body Radiographic Imaging

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the value, benefits, and efficiency of a whole body imaging system as it pertains to radiologic casework in a medical examiner's office, with special emphasis on foreign body localization, skeletal trauma, and scientific identification of remains. Attendees will also be exposed to a variety of other scenarios and circumstances where whole body imaging provides valuable information, including information vital to the safety of morgue personnel.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the many benefits of incorporating a whole body imaging system into a medical examiner's office.

For forensic pathologists, there are many tools available to assist with postmortem examinations. An extremely important tool involves the use of medical imaging technologies. Radiologic findings can be invaluable to the forensic pathologist by providing a tool for radiographic identification, revealing foreign bodies, and uncovering significant pathology. Forensic radiology can be traced back to 1898, beginning with plain films used to localize projectiles. Since then, imaging use has dramatically expanded. Radiography can be used to identify subtle fractures, locate foreign bodies, estimate the biological age of individuals, and help determine the ages of various bony injuries. The uses of imaging modalities have expanded, encompassing not only plain films, but also fluoroscopy and Computed Tomography (CT) scanning.

A relatively recent innovation is the complete body radiographic imaging device. The institution of this study, which is a regional medical examiner's office, obtains at least one full-body scanned image for every case, whether a full autopsy, limited autopsy, or external exam. The scanner is capable of providing a complete body radiographic scan and can perform a complete scan in 13 seconds.

Thus, the scanner can provide a complete, uninterrupted view of the full body, allowing for greater ease of examination and tremendous time-saving. If necessary, multiple scans, including regional and anterior-posterior and lateral views, can be taken to aid in the postmortem investigation. Forensic anthropologists at institutions are responsible for ensuring scientific identification when warranted. The complete body scans are of great value when scientific identification is based on comparative medical radiography.

Several complete body imaging cases will be presented that informed or enhanced the postmortem examination. In addition to providing case examples where complete body imaging has provided excellent and efficient information and necessary documentation for cases, additional examples will be presented that highlight other interesting and sometimes extremely valuable and beneficial information afforded by complete body imaging.

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#### Complete Body Imaging, Complete Body Scans, Radiography

### **H43 Iatrogenic Changes Related to Perimortem Cardiopulmonary Resuscitation (CPR) as Documented by Postmortem Computed Tomography (CT) Scanning**

*Aneesa Baig, MSc\*, Queen Mary University of London, London, England, United Kingdom; Curtis Edward Offiah, MD, Queen Mary University of London, London, England, United Kingdom; Nikolas Petros Lemos, PhD, Queen Mary University of London, London, England, United Kingdom*

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**Learning Overview:** Attendees can expect to learn how to determine which findings may be due to resuscitation-related injury as visualized on postmortem radiological imaging. Attendees will also learn the definition of iatrogenic injury and distinguish this from natural postmortem insults using Postmortem CT (PMCT) scanning. In addition, attendees will learn the intricacies found with postmortem imaging as compared with traditional autopsy, as well as the factors that may cause variations in depth and severity of such injury.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the power of forensic radiology as an aid for the accurate determination of cause of death. The community will also be exposed to natural postmortem changes and be allowed to visually compare those to Cardiopulmonary Resuscitation (CPR) -related injuries.

Although known as a “life-saving” procedure,” forensic pathologists can often find CPR-related injuries that may or may not be related to the primary cause of death. Injuries sustained during perimortem CPR are inevitable due to its intensive nature. With many cases involving resuscitation resulting in death, this study undertook an investigation that aimed to define iatrogenic changes caused by resuscitation methods. Injuries as a result of CPR are commonly encountered at autopsy, but often are found incidentally. When encountering such injuries, care must be taken to define those caused iatrogenically, as similar injuries can be produced for patients with non-accidental injury or even systemic disease. This study inspected PMCT scans from London cases that had been subjected to perimortem CPR. CT imaging is a minimally invasive investigative technique that requires little to no manipulation of the body. Compared with traditional autopsy, the images produced from PMCT imaging can be easily retained and stored, as well as acting as a powerful evidence tool in medicolegal proceedings. Addressing the fundamental concepts of CPR and establishing the injuries that occur from its improper implementation is explored in this study due to the fact that correctly performing CPR can often minimize the severity of injury occurring during resuscitation. This work further helps distinguish between injuries induced from resuscitation compared to those caused by other severe insults, such as abuse or assault.

There have been, however, numerous studies that report the high efficacy of PMCT imaging, and its successful results in determining the causation of death are consistent with the findings of conventional autopsies, thus casting doubt about the need of PMCT analysis. This work offers an understanding of the injuries sustained from resuscitation techniques based on PMCT analysis and discusses the possibility of the injury contributing to the causation of death of an individual.

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#### **Iatrogenic Changes, Postmortem CT Scan, Cardiopulmonary Resuscitation (CPR)**

### **H44 Keeping Up With the Drug/Opioid Epidemic: In-House Testing Program of the King County, Washington, Medical Examiner's Office**

*Jordan Taylor, MD\*, King County Medical Examiner Office, Seattle, WA; Richard Harruff, MD, PhD, King County Medical Examiner Office, Seattle, WA; Nicole Yarid, MD, King County Medical Examiner Office, Seattle, WA; Katie Heidere, MSW, King County Medical Examiner Office, Seattle, WA; Celia Simpson, King County Medical Examiner Office, Seattle, WA*

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**Learning Overview:** After attending this presentation, attendees will be able to explain how a medical examiner office can implement in-house testing for drug overdose deaths to expedite death certification and provide timely, useful data to public health and law enforcement agencies.

**Impact Statement:** This presentation will impact the forensic science community by showing how medical examiners can serve their communities through implementing in-house testing for drug overdose deaths.

The rapid emergence of the drug/opioid overdose epidemic in Washington overwhelmed resources, resulting in extreme delays in receiving toxicology reports. These delays interfered with timely death certification, data collection by epidemiologists, and information provided to law enforcement agencies. As a countermeasure, King County Medical Examiner's Office (KCMEO) initiated "real-time fatal drug overdose surveillance." This presentation reviews the first three full years (2018–2020) of the project.

KCMEO dedicated one medicolegal death investigator, two college student interns, and one statewide Information Coordinator for fatal drug overdose surveillance. KCMEO investigators collected drug evidence at suspected drug overdose death scenes, and KCMEO pathologists selected decedents for in-house testing. Urine from autopsy was tested with a One Step Detect Multi-Panel Forensic test cup, a colorimetric immunoassay dipstick panel of 14 drugs, and a Rapid Response fentanyl test strip. Field portable spectrometers, including Thermo Scientific™ TruNarc™, Rigaku ResQ, and 908 Devices MX908, were used to test drug evidence collected at the scene, and a chemiluminescent immunoanalyzer, Randox® MultiSTAT, was used to screen decedents' blood for 21 analytes. Deaths due to drug overdose were certified based on finding the same drug(s) present in two independent tests, including one from the decedent. Follow-up information was elicited from cooperative family members, and data were provided to law enforcement and public health agencies. All drug deaths certified by this methodology were validated through formal toxicology analysis, and results of drug evidence testing were confirmed by bench laboratory analysis. Data were entered into a Microsoft® Access® database, subsequently migrated to Microsoft® SQL Server linked to the main KCMEO VertiQ database, and analyzed using Microsoft® Excel®.

Over three years, 1,385 drug overdose deaths were certified, 1,092 decedents' blood samples were screened, 1,061 urine samples were tested, and 2,789 tests were performed on drug evidence items from 670 different cases. Drug overdose death certificates were often completed within one day. Overall, the average delay between autopsy and certification of drug overdose deaths decreased from 75 to 31 days. The proportion of all autopsy reports completed in 60 days increased from 55% to 72% and in 90 days increased from 77% to 99%. A collaborative network of local, state, and federal law enforcement and public health agencies was established, and weekly and monthly bulletins were published. To date, 25 of the 39 Washington counties participate in the surveillance project.

Although deaths due to drug overdose continue to accelerate, KCMEO became an active participant in controlling the epidemic by providing timely and usable intelligence informing and evaluating response efforts through this program. The fatal drug overdose surveillance program developed into a collaboration between public health and law enforcement agencies. As the drug/opioid overdose epidemic has been declared a "public health emergency," it is appropriate that medical examiners feel compelled to participate in combating the epidemic. The experience of KCMEO validates such efforts and serves as a model for other offices.

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#### **Drug Overdose Surveillance, Postmortem Toxicology, In-House Drug Evidence Testing**



### H45 An Internal Validation of the ANDE® 6C Expert System for the Analysis of Unidentified Human Remains in a Forensic Laboratory Setting

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**Learning Overview:** Attendees can expect to see the results of the internal validation on Rapid DNA identification performed on several tissue types, including blood, muscle, liver, hair, tooth, and bone samples for the implementation of Rapid DNA as a viable technology for the analysis of Unidentified Human Remains (UHRs) in a forensic laboratory setting.

**Impact Statement:** The use of a Rapid DNA instrument for the analysis of evidentiary forensic casework samples, with or without human intervention, is not an approved use of this technology at this time. This presentation seeks to help those forensic laboratories that assist a medicolegal client in the identification of unidentified human remains, see the benefits of using this technology for these purposes in an accredited laboratory setting, and explore the possibility of analyzing other types of samples as well.

The Rapid DNA Act (H.R. 510) became United States federal law on August 18, 2017. The approval of this law permits criminal justice agencies and forensic science providers to use Rapid DNA instruments approved by the Director of the Federal Bureau of Investigation for their use in the testing of reference samples without human intervention at police booking stations or in an accredited laboratory setting. However, the use of a Rapid DNA instrument for the analysis of evidentiary forensic casework samples, with or without human intervention, is not an approved use of this technology at this time.

The Forensic DNA-Serology Laboratory of the Puerto Rico Institute of Forensic Sciences (PRIFS) is the only forensic laboratory in Puerto Rico to analyze all forensic cases such as homicides, sexual assaults, and more. Part of the services offered by this laboratory is the identification of UHRs requested by the Medical-Legal Division. These identification cases can take up to 60% of the analyst's time, which could otherwise be used to solve other crimes. The objective of this study was the internal validation of the ANDE™ 6C Expert System for a Modified Rapid DNA Analysis of UHR samples using the I-Chip (Part number A0210001060) designed for forensic samples.

The ANDE™ System consists of automated purification, amplification, separation, detection, allele calling, and Expert System interpretation. The use of a Rapid instrument for identification cases will allow the PRIFS to analyze UHRs and their reference samples in an expedited manner. UHRs not identified or with unacceptable results would later be analyzed using conventional DNA methods and technologies for Combined DNA Index System (CODIS) upload. A total of 114 blood, liver, muscle, buccal, hair, tooth, and fresh and degraded bone samples were analyzed to assess the suitability of the ANDE™ 6C System for use with I-Chips and human intervention as Modified Rapid DNA. Internal validation studies included sensitivity, accuracy/concordance, reproducibility, sample-to-sample contamination, 1:1 and 1:5 mixtures, mock samples, and a certified reference material, focusing on viability for use in the identification UHRs. All samples were run in duplicate for reproducibility. Sensitivity studies included 1µL, 5µL, 10µL, 20µL, 40µL, and 80µL of blood, and blood dilutions of 1:1, 1:4, 1:10 1:20, and 1:100.

The ANDE™ 6C Expert System Disabled Setting with 12 passing autosomal was used, which identifies samples with low DNA and applies a less conservative calling algorithm, allowing one to use and evaluate more data generated by the instrument.

Reproducibility of results averaged at 96% with loci designated as passing loci. Loci designated as not pass was due mostly to complete loci dropout; 99.66% of alleles in passing loci were determined to be true alleles. Dropout of sister alleles in true heterozygote loci was observed in 1.33% of the samples. This phenomenon occurred in loci with less than 6,000 Relative Fluorescence Units (RFU). Thus, 98.67% of homozygote alleles below 6,000 RFU can confidently be considered homozygote alleles. In contrast, 52 out of 149 (35%) heterozygote and Y-chromosome loci deemed as "not passing" demonstrated concordance with reference sample. These internal validation results led to the approval of the ANDE™ 6C Instrument together with the single-use I-Chip consumable performing the FlexPlex™ assay for the analysis of UHR samples in the PRIFS.

#### Rapid DNA, Unidentified Human Remains, Forensic Laboratory

### H46 Forensic Photography—An Invaluable Tool During Forensic Autopsies

*Rachael Landrie, BS\*, Office of the Chief Medical Examiner, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will learn the process of photo documentation during forensic autopsies as well as how to stage the body and organs in order to capture high-quality forensic images and achieve favorably presentable photographs.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating invaluable processes regarding forensic photography that can be utilized during forensic autopsies.

A forensic autopsy is performed in a medical examiners or coroner's office and, typically, the autopsy is documented by the forensic pathologist by a written report. The external exam involves documenting the body for any scars, trauma, or disease, as well as identifying features such as hair color and length, tattoos, eye color, etc. Internally, the organs are evaluated for trauma and disease. These findings as well as toxicology and histology are used by the forensic pathologist to determine cause and manner of death and are included in the final autopsy report. Photographs taken at the time of autopsy can serve to further document important and pertinent findings visually.

Frequently, forensic photography is used to provide detailed, visual documentation of forensic investigations, including crime scenes and physical evidence not limited to fingerprints, shoe mark impressions, and shell casings for legal purposes; however, forensic photography can also play an important role in visually recording the body during a forensic autopsy. The body is physical evidence. Photo documentation provides a visual explanation of what is documented in the autopsy report, which may be difficult to describe verbally. As a result, forensic pathologists can review these photos to support their autopsy report and recall detailed information about the case if called to testify in a court of law. Other uses allow for forensic pathology teaching, consultations, and, importantly for attorneys, to present as evidence during court trial proceedings.

With advances in digital technology, capabilities of forensic photography have increased exponentially. Digital photography equipment is more user friendly and affordable, photographic supplies are readily available, and digital photographs are easy to store and display. Through mindful attention to decedent and organ preparation, composition, and basic camera/lighting settings, forensic autopsy photos can be achievable accurately and quickly. Current methods in the use of digital equipment and supplies, basic autopsy photography flow, and examples of acceptable versus unacceptable photographs will be presented to support the significance of photography in the forensic community.

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#### Autopsy, Photography, Pathology

## H47 The Logic of Forensic Pathology Opinion

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how Bayes' theorem can help forensic pathologists provide logically sound and transparent evidence, while staying within their areas of expertise.

**Impact Statement:** This presentation will impact the forensic science community by discussing the benefits of applying Bayes' theorem and providing advice on how to apply it in forensic pathology.

Evaluating evidence, and providing opinions based on evidence, are subjects of ongoing importance within all forensic sciences. Among others, forensic experts are expected to provide evidence that is based on logically sound and transparent scientific reasoning that honors the boundaries of their area of expertise. In order to meet these objectives, many fields of forensic science explicitly use Bayes' theorem when expressing their opinion. This theorem describes the logically correct way to update probabilities on the basis of observations and is therefore well-suited for forensic evidence interpretation. The benefits are numerous. Among others, it helps expert to stay within their remit, increases the relevance and specificity of the expert opinion, and provides clarity about the roles of the expert and the trier-of-fact.

Making a distinction between “investigative” and “evaluative” modes of operating helps to implement the theorem into daily casework. The distinction between the two recognizes that the amount of information that is available to experts changes over time, and that this affects their roles and the type of statements they can make.

Despite these developments in other fields of forensic science, forensic pathology seems slow to adopt these principles. As a consequence, the majority of forensic pathology reports currently do not meet the objectives stated above. This presentation explores this issue further and suggests a way forward.

This presentation starts with a short introduction into Bayes' theorem and its benefits, followed by a discussion of why its application is actually second nature to medical practitioners. It then discusses the difference between investigative and evaluative opinions, and how they enable the forensic pathologist to apply Bayes' theorem in the different phases of a forensic investigation. Several practical examples will be used to support this presentation.

The overarching aim of this contribution is to advance the implementation of logically correct reasoning and reporting in forensic pathology.

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### Forensic Pathology, Evidence Interpretation, Likelihood Ratio

### **H48 Work-Related Fatal Injuries in the Genoa District of Northwest Italy: A Forensic Analysis of the 2011–2020 Decade**

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**Learning Overview:** After attending this presentation, attendees will have learned about the occupational-related deaths; a worldwide increasing problem with severe social repercussions. In particular, this presentation aims to bring the attention of the forensic science community to the relevance of deaths that occurred in the workplace and were forensically investigated at the Institute of Forensic Medicine of Genoa from 2011 to 2020.

**Impact Statement:** This presentation will impact the forensic science community by underlying the aim of providing a forensic and medical-legal framework of fatal accidents during work.

According to the International Labor Organization, an occupational accident is an incident that occurs in the course of work and results in a fatal or non-fatal injury. In the Italian scenario, the first cause of fatal accidents at work includes road accidents, crushing and falls from above, followed by other causes in smaller numbers, including burns, electrocution, suffocation and asphyxiation, drowning, and explosions.

In Italy, the sectors most affected are industry and agriculture, while the prevailing sex is male: in particular for fatal accidents, men are affected ten times more often than women, for a total of 4,197 cases, including 273 deaths. In recent years, the social phenomenon of immigration has also taken on an increasing importance in the field of accidents at work; there is, in fact, an increasing presence of foreign workers in the Italian labor markets, especially the strictly manual labor market, and their increasing involvement in accidents.

The data analyzed for each death shall include the nationality and sex of the worker, the employment performed at the time of death, the year in which the fatal event occurred, the time slot, the day of the week and the month in which the event occurred, the use of personal protective equipment, any medical treatment carried out on the subject (from immediate resuscitation procedures to subsequent hospitalization), the cause of death and the prevalent injuries and, finally, the results of toxicological tests.

This study includes a list of 47 people that have died because of an occupational injury: 46 males and only 1 female. The construction worker/metalworker was the most exposed to fatal accidents (40.5%). In 41 cases (87.2%), the death was related to a major mechanical trauma secondary to precipitation (42.5%), falling objects directly on the body of the victim (38.3%), or pedestrian road investment (6.4%). A fatal head trauma with endocranial hemorrhage occurred in 23 cases (63.4%). In 4 out of 47 cases (8.5%), death was due to a violent asphyxiation mechanism, of which 3 were drowning and 1 was accidental hanging. Finally, the last 2 deaths were caused by an electrocution mechanism.

In conclusion, fatal accidents at work almost exclusively affected men, consistent with national and international cases. However, men are also employed in more dangerous and tiring work tasks, with a higher risk of accident and death, while women are more obedient to safety rules. The work of a construction worker/metalworker remains the one with the greatest risk of accidents at work: often, in fact, they are precarious occupations, carried out by subjects employed in part-time or temporary and contract positions, with rights and safeguards much more limited than those held by the majority of the employed population, despite the high occupational risks.

Moreover, this study highlights the importance of the fact that, although high-income countries, thanks to surveillance, reporting, and survey methods and the changing of legislation over the years, have achieved a reduction in deaths at work, the number of fatal accidents in construction, industry, and shipbuilding remains high.

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#### **Work Accident, Fatal Injury, Mechanical Trauma**

### H49 Diving Accidents and Related Deaths in the Genoa District of Northwest Italy: A Forensic Analysis of the Period From 1968 to 2020

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**Learning Overview:** After attending this presentation, attendees will have learned important aspects about the causes of death resulting from scuba diving accidents during the past 52 years in the City and Province of Genoa (1968–2020). In particular, this presentation will aim to bring the attention of the forensic science community to the relevance of increased fatal accidents brought about by the introduction of easy-to-use scuba diving equipment and the consequent widespread increase of scuba diving activity in the Genoa area.

**Impact Statement:** The presentation will seek to impact the forensic science community by emphasizing the description of the forensic medical approach in fatal diving accidents based on analyzing the characteristics of the population involved, the circumstantial data, and the outcomes of the investigations performed.

The Province of Genoa, having a mainly rocky coastline, attracts many seashore tourists of many different nationalities. Scuba diving is divided into recreational and technical diving, depending on the time, depth, and breathing mixtures used (pure oxygen, nitrox, trimix). According to literature, most deaths during diving activities are caused by drowning; however, the primary cause of death is different.

The present study included 48 subjects who died because of scuba diving accidents; 44 males and 4 females. All subjects underwent autopsy examination and histological investigations, toxicological screening, and equipment analysis, as applicable. Also analyzed were the characteristics of the population, the circumstances of the event, and the data from the investigations. In 34 subjects, the type of diving was known: 16 recreational and 18 technical.

Regarding the breathing mixture used, 22 subjects used compressed air breathing apparatus, 4 nitrox, 3 pure oxygen, 3 trimix with Enriched Air Nitrox (EAN), 1 trimix, and 1 air with nitrox. Seven subjects were using rebreathers. The dive period, in which the diving accident occurred, is known only in 26 cases: 10 during the descent, 14 during the ascent, and 2 post-dive.

Of the 48 analyzed incidents, 7 were attributed to technical factors and 3 to human error. The histological study showed in 26 subjects predisposing pathological factors; mainly cardiovascular. The most common pathological findings were: polyvisceral congestion (83.3%), pulmonary edema (77.1%), pulmonary emphysema (72.9%), “foamy fungus” (47.9%), rupture of the interalveolar septa (45.9%), conjunctival petechiae (45.9%), and subpleural petechiae (33.3%). In 12 diving-related fatalities (25%), the identified cause of death was exclusively drowning, whereas in the other subjects the primary causes of death were identified in: Arterial Gas Embolism (AGE) following pulmonary barotrauma (27.1%), cardiocirculatory arrest due to heart attack (16.7%) or other causes (8.3%), confined space asphyxia (12.5%), gas intoxication (4.1%), Decompression Illness (DCI) (4.1%), and AGE and DCI combined mechanism (2.1%).

The analysis of the data showed that, in less recent cases, the cause of death was attributable to mechanical asphyxia secondary to drowning. Later, thanks to the improvement of diagnostic techniques and knowledge in the field of diving medicine, according to literature, it was possible to determine the specific primary cause of the diving accident.

In conclusion, from data obtained, fatal accidents could be limited by more accurate medical controls and the introduction of periodic refresher courses. Moreover, this study highlights the importance of the role of medical examiner to clarify the circumstances of death and to allow the continuous increase of safety in the practice of this activity, expanding the knowledge in the field of diving medicine.

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#### Scuba Diving, Fatal Accident, Drowning

## H50 An Unusual Gunshot Wound Suicide With Findings Mimicking a Second Entrance Wound

Harrison Moosavi, MD\*, Georgia Bureau of Investigation, Decatur, GA, and Augusta, University–Medical College of Georgia, Augusta, GA; Andrew Koopmeiners, MD, Georgia Bureau of Investigation, Decatur, GA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of correlating scene findings, investigative details, and autopsy findings for proper interpretation and certification of suicidal gunshot wounds.

**Impact Statement:** This case will demonstrate an unusual method of suicide by using handguns simultaneously to inflict gunshot wounds of the head, with only one firearm successfully firing, but two wounds being present.

Suicide is the tenth leading cause of death in the United States. In 2019, 50.4% of the 47,511 suicides in the United States involved firearms.<sup>1</sup>

The presented case is of a woman who committed suicide by simultaneously holding two handguns against opposite sides of her head.

A 53-year-old woman was found supine in her bed with a .380 semi-automatic handgun in her right hand and 9mm semi-automatic handgun between her left arm and left side of her chest. One spent .380 shell casing was located at the scene. Two unspent 9mm rounds were found on the floor near the bed. Both weapons had multiple unspent rounds in their magazines. Both weapons contained tissue, blood, and head hair on the ends of the barrels.

At autopsy, a contact gunshot wound was identified on the right temple with an associated abrasion consistent with a muzzle imprint, with a bullet track passing through the skull and brain with no associated exit wound. There was a laceration of the left temple with an associated patterned abrasion consistent with a muzzle impression; however, there was no gunshot wound associated with this defect. Per scene photography and investigative information, it was revealed that only the .380 handgun discharged. The cause of death was a gunshot wound of the head.

Suicides involving the use of multiple firearms are uncommon, but not unheard of. Review of the literature and case reports involving multiple firearm suicides usually involve simultaneous gunshots to the head and chest, or head and abdomen.<sup>2,3</sup> Additional cases of multiple gunshot wound suicides involve defective firearms in which the initial gunshot was insufficient to cause death or incapacitation and subsequent gunshots were fired.<sup>4</sup> Only one other similar case from 2019 involving simultaneous attempted gunshot wounds of the head with only one firearm successfully discharging was reviewed.<sup>5</sup> This case represents an unusual circumstance in which the laceration and associated abrasion caused by the head striking the barrel of an unfired handgun mimics a second entrance wound.

This case highlights the importance of correlating scene findings, investigative details, and autopsy findings to allow for proper interpretation and certification of suicidal gunshot wounds.

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### Multiple Firearm Suicide, Gunshot Wound Suicide, Gunshot Wounds of the Head

**H51 A Study on the Suicide Statistics Using Sodium Nitrite in Korea Over the Past 10 Years (2011–2020) and on the Identification Method of Sodium Nitrite Using Coloration Reaction Reagents at the Unnatural Death Scene**

*Jin Yeong Kim, MD\*, Korea National Police Agency, Seoul, Korea*

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**WITHDRAWN**

## H52 A Tale of Two Suicides—Gunshot Wounds to the Occiput

Jiemin Zhou, MD\*, Office of the Chief Medical Examiner, Baltimore, MD; Zabiullah Ali, MD, Office of the Chief Medical Examiner, Baltimore, MD; Stephanie Dean, MD, Office of the Chief Medical Examiner, Baltimore, MD; J. Laron Locke, MD, Office of the Chief Medical Examiner, Baltimore, MD

**Learning Overview:** The goal of this presentation is to alert attendees to not presume manner of death from atypical gunshot wounds of entrance.

**Impact Statement:** This presentation will impact the forensic science community by highlighting two cases involving self-inflicted gunshot wounds to the back of the head.

**Case 1:** This 28-year-old man was found prone in the water of the Patapsco River in central Maryland. He had originally texted his girlfriend stating he had a flat tire. The girlfriend then went to the incident location, found the decedent, and alerted emergency services. At the scene, a Glock® 26 was found in the river next to the decedent's head. His truck was in the parking lot with a flat tire. An empty gun holster was attached to the front dashboard. Additionally, the truck appeared ransacked with a wallet on the ground with missing money. A bullet hole was also noted in the tailgate. Against the truck were a spare tire, tire jack, and tire iron.

Autopsy revealed a single contact gunshot wound to the back of the head near the parietal-occipital region. Soot was found on the scalp, subgaleal surface, and underlying bone. An exit wound was located on the left side of the forehead with recovery of projectile fragments along the wound path. Postmortem toxicology testing of the heart blood was positive for alcohol (0.17% w/v).

Further investigation revealed the decedent had a history of drug and alcohol abuse. He had a recent argument with his girlfriend in which he later sent a vague, cryptic text-message to her. The decedent also complained of career-related difficulties. There were no signs of a struggle; nothing was stolen from the decedent; and no other tire tracks were in the parking lot. Based on investigation, the manner of death was ruled a suicide.

**Case 2:** This 76-year-old man was found rolling in the surf line at Ocean City, MD, when he was then brought ashore. A Ruger® belonging to the decedent was located a short distance away days later by a person searching for metal with metal detector on the beach.

Autopsy revealed a single gunshot entrance wound to the right occipital scalp with gunpowder stippling extending up to ½" from the entrance wound. The bullet did not enter the cranial cavity but did cause subarachnoid hemorrhages and cerebral contusion. Multiple bullet fragments, imbedded in the skull, were recovered; the bullet exited the right occipital scalp, approximately 1" from the entrance wound. The autopsy was otherwise remarkable for immersion changes; heavy and edematous lungs; aqueous fluid in the sphenoid sinus; and findings suggestive of prostate carcinoma. Toxicologic analysis was negative for alcohol and drugs.

Further investigation revealed video recording of the decedent walking to the beach, then disappearing before the next street camera. According to his family, he was "hiding an illness" and had lost 50 pounds in the last six months. In his residence, his cell phone, keys, and all important documents and papers were organized and placed on the kitchen table. Based on investigation, the manner of death was ruled a suicide.

**Discussion:** Firearms are used in the majority of suicides in males in the United States. Gunshot wounds to the occiput comprise less than 1% of suicides. Staged homicides, such as the arranged scene in Case 1, may further obfuscate the course of events. While the location of the entrance wound may suggest homicide, the importance of a thorough investigation and collection of evidence cannot be overstated in determining the proper manner of death.<sup>1,2</sup>

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### Suicide, Self-Inflicted Gunshot Wounds, Back of Head



## H53 Deaths Associated With SARS-CoV-2 Vaccination: The Importance of Autopsy

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**Learning Overview:** Following this presentation, attendees will understand how the autopsy should be a rule in the causality assessment of fatal cases temporally related to COVID-19 vaccination.

**Impact Statement:** This presentation will impact the forensic community by providing up-to-date data about the pivotal role of forensic sciences providing scientific evidence to dissolve the doubts of public opinion regarding COVID-19 vaccination.

The current challenge worldwide is the administration of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine. To date, the European Community, following the recommendation by the European Medicines Agency (EMA), has authorized the use of four vaccines. The vaccine of the company BNT162b2 (Pfizer®–BioNTech®) was authorized on December 21, 2020; the second mRNA-1273 (Moderna®) was approved on January 6, 2021; the third vaccine, ChAdOx1 nCov-19 (AstraZeneca®), was approved on January 29, 2021; and the last vaccine is COVID-19 Vaccine Janssen (Johnson & Johnson®), authorized on March 11, 2021.<sup>1-4</sup>

Nevertheless, severe adverse reactions temporally related to vaccine administration have generated diffidence in the population, slowing the European vaccine plan.

This communication aims to analyze the pivotal role of forensic sciences in order to define the causal relationship between Adverse Events Following Immunization (AEFI) and COVID-19 vaccination. To achieve this goal, a literature review was conducted using the following keywords: “COVID-19 vaccination,” “Post-mortem findings,” and “autopsy findings” in different databases (PubMed®, Scopus®, Web of Science®).

Although different fatal events temporally related to the COVID-19 vaccine administration were reported, the postmortem investigation has been performed in a few cases (nine papers have published fatal cases temporally related with COVID-19 vaccine administration, discussing about 16 cases). Based on this data, autopsy is very useful to define the main characteristics of the so-called Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) after ChAdOx1 nCoV-19 vaccination: the recurrent findings were intracranial hemorrhage and diffused microthrombi located in multiple areas.

Vaccines represent some of the greatest medical and scientific achievements of the modern era. Particularly in the pandemic scenario, a medicolegal perspective on vaccination is very important in order to provide a critical viewpoint. The COVID-19 vaccine administration involves different questions, such as the possibility of side effects, which led to a fairly diffuse suspicion and rejection, especially when a fatal case temporally related to vaccine administration occurs in a young healthy subject. The use of vaccines also poses various ethical and legal problems, including the possibility of conflicts between individual and collective rights.<sup>5,6</sup>

The great challenge for the scientific community in the fight against COVID-19 is represented by the success of a global vaccination campaign and, in this light, it is important to provide scientific evidence to dissolve the doubt of public opinion. In order to avoid another “Lockdown of Science,” the autopsy should be a rule in the causality assessment of fatal cases temporally related to COVID-19 vaccination.<sup>7</sup>

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### Forensic Science, COVID-19 Vaccination, Adverse Events Following Immunization (AEFI)

### H54 The Time and Temperature Dependence of Volatile Organic Compounds' (VOCs') Appearance in Autolytic Rat and Pig Liver

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**Learning Overview:** After attending this presentation, attendees will better understand the potential for VOCs to provide information regarding Postmortem Interval (PMI).

**Impact Statement:** The goal of this research is to explore the pattern of volatile compound generation as an indicator of time since death during the early stages of decomposition.

The PMI reflects the period between death and the discovery of a body. In some cases, an accurate estimation of PMI may play a significant role in death-related investigations. Current methods for the determination of PMI are primarily subjective and based on observation of the decedent and their surroundings. During decomposition, endogenous enzyme activity produces a number of VOCs characteristic of the process. Much of the available literature describing postmortem production of VOCs is focused on the later periods of decomposition, during which extensive microbial activity produces many of these compounds. This project has focused on the autolytic period that occurs before breaching of the Gastrointestinal (GI) tract, and the extensive microbial colonization of the body that follows, initiating the putrefactive stage of decomposition. This work explores the time- and temperature-dependent relationship between specific volatiles and the potential of such relationships to correlate to a particular range of PMI. In using the ratios of specific target VOCs on a time- and temperature-dependent model, details on the PMI may be elucidated.

Incubations of a rat liver homogenate at two different temperatures (room temperature and 28°C) were conducted for five-day periods, with triplicate aliquots removed at 0, 6, and 12 hours, and at 12-hour intervals thereafter. Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS) analysis was used to identify specific compounds generated at each time point. The relative abundance of detected compounds was then plotted against time and temperature. Similar experiments were performed using pig liver.

Compounds of interest in the rat incubations were acetaldehyde, ethanol, acetone, acetoin, and 3-methyl butanal. Ethanol, acetoin, and hexanal were similarly found in pig liver. A linear temporal relationship between Acetone (A) and Ethanol (E) in the room temperature incubation of rat liver was determined, expressed as:  $A/E \text{ Ratio} = -0.0104(\text{Time}) + 0.08066$ , yielding an  $R^2 = 0.97$ . Thus, for these compounds, under these specific experimental conditions, the A/E ratio appeared to be highly correlated to PMI for the 24–72-hour incubation period. Acetoin-to-acetaldehyde also appeared to have a linear relationship. In contrast, the hexanal-to-acetaldehyde, and acetone-to-acetoin ratios appeared to be exponential.

Despite the lack of consistency between the apparent nature of the relationship between specific analytes, be it linear or exponential, these findings suggest that the ratio of specific volatiles with different temporal evolution profiles may be useful for the determination of PMI.

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#### Postmortem Interval (PMI), Volatile Organic Compounds (VOCs), Decomposition

### H55 A Complex Suicide by Drowning and Stabbing: The First Italian Case Reported in the Literature

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of distinguishing this kind of complex suicide from others or from violent forms of death, such as homicides or accidents.

**Impact Statement:** This presentation will impact the forensic science community by presenting the first case in Italy of a complex suicide through self-stabbing and drowning.

The term “complex suicide” refers to suicides in which more than one suicide method is applied.<sup>1</sup> In a planned complex suicide, the victim plans different and simultaneous methods of death, to prevent one of them from failing. In an unplanned complex suicide, on the other hand, the victim changes the method because the first one fails or turns out to be too painful.

The case presented is the first Italian example reported in major literature of an unplanned complex suicide achieved through stabbing and drowning by a man with no psychiatric history or alcohol/drug abuse history.

The body was found lying on the front seats of a car, in a field outside the city, near the seaside. The clothes worn by the corpse were intact, wet, and smeared with mud and gravel compatible with the characteristics of the shoreline leading to the sea. Inside the car, a kitchen knife with a serrated blade and a pair of scissors were found, both smeared with dried blood material.

The cadaveric inspection showed multiple superficial stab and cut injuries. The leakage of aqueous-like transparent liquid from the nasal cavities was also found. The external examination of the corpse confirmed the presence of multiple stab lesions, mostly punctiform, some of them triangular in shape and others linear, mostly superficial, located in different body sites: head, neck, chest, and abdomen. None of the internal organs or blood vessels were affected. Numerous superficial linear excoriated lesions were also investigated at the level of the right and left latero-cervical regions of both wrists. There were no outcomes of violent or defensive injuries attributable to the actions of third parties.

At the opening of the thorax, at the level of the left hemithorax, there was extensive hemorrhagic infiltration of the subcutaneous soft tissues and muscle tissues, as well as two infiltrations in correspondence with the sternal manubrium and the left parasternal region. Upon examination of the lungs, these were expanded, heavily congested, with increased volume. On the section surface was observed the leakage of liquid material mixed with air. The blood and urine taken during the autopsy examination were used to carry out toxicological investigations, which gave negative results. The cause of death was attributed to hypothermia in a subject with multiple stab wounds and evidence of incomplete drowning.

The case described above is the third case worldwide and the first case in Italy of complex suicide through self-stabbing and drowning.<sup>2,3</sup> In cases of suicide, it is necessary to analyze all the evidence, which makes possible to distinguish this kind of sudden/unnatural death from others or from a violent form of death, such as homicide or accident. In these cases, it is mandatory to perform an accurate external examination of the corpse, an autopsy, and lab tests in order to ascertain the cause of death with certainty. Moreover, a multidisciplinary approach including a psychiatric criminological investigation could help in finding the truth of how and why the suicide was prepared.

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#### Complex Suicide, Drowning and Stabbing, Italian

## H56 Spectrophotometric Analysis of Postmortem Lividity: A New Objective Measure to Define Time of Death

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**Learning Overview:** After attending this presentation, attendees will be aware of a new and innovative method for hypostases objective detection that can reliably define the time since death.

**Impact Statement:** This presentation will impact the forensic science community by presenting a new instrumental analysis procedure based on a spectrophotometric technique that provides a promising way of interpreting the hypostatic phenomenon with high reliability.

The correct definition of time of death often represents a key to solving judicial cases. However, a problem frequently encountered in medicolegal practice is the difficulty of precisely defining the “Time Since Death” (TSD).<sup>1-3</sup> One of the thanatochronological parameters used in forensic medicine is the study of hypostases and their characteristics in relation to the migration and the possibility of using digital compression to highlight the changes they undergo in the different periods after death. However, these are based on non-objective parameters, and are therefore characterized by an intrinsic variability that can compromise their reliability. In fact, as the results of numerous worldwide studies have shown, the current traditional methods of assessment of hypostases for thanatochronological purposes are characterized by numerous limitations and, therefore, they are not conclusive.<sup>4-7</sup>

The purpose of the present study was to propose a standardized method of objective detection of hypostases, which still represents a weak link in the thanatochronological triad used, thus allowing estimation of time since death with good approximation. To achieve this, the instrument used was based on spectrophotometry in order to analyze only the hemoglobin contribution to skin color, while excluding the role of melanin.<sup>8,9</sup> This made it possible to analyze a hypostatic area, with a numerical value defined as a measure of the hemoglobin content before and after application of standardized pressure for a defined time interval. The study was conducted on 100 cadavers in various wards. The variables considered in the study were: Hb-pre (mean hemoglobin measure before weight force application); Hb-post (mean hemoglobin measure after weight force application),  $\Delta$ Hb (change in mean hemoglobin measures from before to after weight force application); and red% Hb (percentage reduction in hemoglobin after weight force application, compared to the mean measure of hemoglobin before weight force application).

The data analysis showed statistically significant correlation between the red% Hb value, as the percentage reduction of post-compression hemoglobin, and time, in terms of time since death. This provided a mathematical formula to estimate time since death with good reliability ( $\pm 4$  h, 50 min).

The spectrophotometric technique used has proven to be valid and effective for the evaluation of hypostases, mainly because it can provide the operator with quantitative data for the hemoglobin levels in the skin scans analyzed, a characteristic not found in any other study in the literature to date. This method also applies to the potential for more in-depth evaluation of the biological factors that influence the phenomenon analyzed, essentially with a view to attribute these to specific causes of death. Finally, a potentially revolutionary aspect of the proposed technique that will be evaluated in future studies lies in the possibility of analyzing the cutaneous quantity of hemoglobin also in the skin of subjects of Negroid ethnicity, therefore overcoming this objective limitation in the analysis of hypostases in such cadavers.

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### Time Since Death, Spectrophotometric Analysis, Postmortem Lividity

### H57 The Effect of Multiple Washes and Thread Count on Semen-Stained Sheets

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**Learning Overview:** After attending this presentation, attendees will better understand how the weave and thread count of fabric affects the retention of sperm cells and proteins in a semen stain after laundering. Attendees will also learn how sperm cells and other materials were retained following repeated washings up to ten times.

**Impact Statement:** This presentation will impact the forensic science community by presenting scenarios where semen stains on articles of laundered clothing or bedding after a sexual assault may be difficult to locate, but may still contain sperm cells capable of producing a complete DNA profile.

Since its development, DNA analysis has been the gold standard for crime scene evidence. In cases of sexual assault, DNA evidence such as seminal fluid is extremely valuable. It may be the only evidence left at the scene that can link the perpetrator to the victim. Analysts have explored the possibility of obtaining DNA profiles from biological evidence from stained fabrics that have been laundered.

Successful extractions are dependent on the conditions used when laundering the fabrics. Wash temperature, chemicals and detergents, number of washes, and fabric structure can affect how long a stain remains on a piece of fabric. Previous studies have focused on temperature settings, types of detergents and chemicals, and drying in a machine dryer versus air drying. In general, fluorescence and acid phosphatase are washed away more easily than sperm cells. In this project, semen stains were deposited on bed sheets and laundered. The thread count of sheets were varied along with the number of washes in a washing machine. This study focused on how the weave and thread count of material affects the persistence of a semen stain and how multiple washings diluted the stain until an attempt to obtain a DNA profile failed. Utilizing regular wash and dry cycles simulated what may occur to evidence after a crime and thus could provide information on whether analysts can use articles of clothing or bedding after a sexual assault even if those items have been laundered multiple times. Overall, data indicated that fluorescence could be removed following a single wash, but sperm cells remained following multiple washes.

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#### Laundered Semen Stains, Thread Count, DNA

## H58 The First Italian Suicide Death Due to Helium Inhalation: The First Histological Findings and Pathogenesis

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**Learning Overview:** After attending this presentation, attendees will be able to recognize the histopathological data of the lungs in deaths caused by helium intoxication

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from a combined death's mechanism: helium asphyxia and asphyxia by obstruction of the airway.

Every year in Italy, about 4,000 suicides are documented accordingly to The Italian National Institute for Statistics (ISTAT). Suicide rates are higher among older people; among young people, however, suicide is, in Italy as in the rest of the world, one of the first causes of death.<sup>1</sup> According to the literature, not many cases of suicides by helium gas have been recorded recently. The continued inhalation of helium may induce loss of consciousness within 5-10 seconds and lead to hypoxic death in few minutes.<sup>2,3</sup> The "procedure" of this suicidal technique, including the so-called "suicide bags," is well described on many easily accessible websites; the above-mentioned bags were indeed used in the first cases reported in literature.<sup>4</sup> These consist of a kit composed of a plastic bag connected and fixed by a drawstring or adhesive tape to a plastic tube linked to a gas bottle. The plastic needs to be hermetically sealed, so it is usually also fixed tightly to the neck.

A 28-year-old Italian man was found dead in a hotel room. The corpse was lying on the bathroom floor. His head was wrapped in a plastic bag, closed around the neck with a tape, and connected to a 2.2L helium cylinder by a plastic tube. The external examination showed a ligature mark of the skin that resulted from the compression of the neck by the tapes and the elastic. Autopsy revealed only a marked and widespread congestion and cerebral edema, expanded and big lungs, with reddish petechiae bilaterally. On cut-section, the lung parenchyma was pale, with emphysematous-like aspect and a foam-like structure. Based on these findings, death was attributed to a double asphyxiating mechanism: helium intoxication and asphyxia in a confined space due to the plastic bag wrapped around the head. It was not possible to measure the helium concentration in the blood sample because helium can not be detected using standard analysis.

The case presented here is the first case reporting the microscopic data of helium inhalation. Asphyxiation by helium is a very rare occurrence and, per research, only few cases are reported in literature. A combined mechanism is seen in this case: gas asphyxia and asphyxia by obstruction of the airway are both present. The plastic bag alone may have been sufficient to cause asphyxiation even without helium, but the addition of the gas allowed a more rapid loss of consciousness and death.

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### Asphyxia in a Confined Environment, Helium Intoxication, Combined Death Mechanism

## H59 Postmortem Findings in 11 Cases of Deaths Occurring After the Administration of an Anti-SARS-CoV-2 Vaccine

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**Learning Overview:** The goal of this presentation is to describe and explain the macroscopic and microscopic postmortem findings in 11 individuals who died following administration of the anti-SARS-CoV-2 vaccine.

**Impact Statement:** This presentation will impact the forensic science community by underlying the key role of postmortem investigations in the clarification of disorders related to the SARS-CoV-2 vaccine to improve vaccine safety.

During 2020 and 2021, the global pandemic of COVID-19 has resulted in high death rates and acute and chronic morbidity worldwide. Large-scale anti-SARS-CoV-2 vaccination campaigns brought hope that the spread of this virus could be controlled; to date, the European Community, following the recommendation by the European Medicines Agency, has authorized the use of four vaccines: Pfizer®-BioNTech® “Comirnaty,” Johnson & Johnson® “Janssen,” Moderna® “Spikevax,” and AstraZeneca® “Vaxzevria.”<sup>1,2</sup> During the pharmacovigilance phase, for millions of people who received the new vaccines, mild side effects, such as fever or pain at the injection site, were frequently reported; however, in sporadic cases, severe side effects were also reported.

In this study, as well as in other reports in literature, most of the deaths occurred shortly after the vaccine administration and were due to thrombosis and its consequences; therefore, it is important to ascertain if administration of the abovementioned vaccines, with particular individual susceptibility, could cause alterations in blood aggregation and coagulation, leading to a fatal outcome.<sup>3</sup>

**Materials and Methods:** This study has been conducted on 11 subjects who died from February to July 2021 after administration of anti-SARS-CoV-2 vaccines. The subjects were between 18 and 86 years-old, 7 females and 4 males: 4/11 (36%) died after receiving the Vaxzevria (after the second dose), 7/11 (64%) after the Comirnaty (3/7 after the first dose; 4/7 after the second one). Death occurred within 1 hour to 16 days after the administration of the vaccine.

Each subject underwent a forensic autopsy at the Legal Medicine Unit of the University of Pavia, followed by histological examination. Samples taken from the brain, heart, lungs, liver, spleen, kidney, blood vessels, and thrombi (if present) were routinely fixed in 10% formaldehyde solution for at least 24 hours and stained with hematoxylin-eosin.

In addition, an immuno-histochemical staining was performed: anti-CD42 and anti-PF4 antibodies were used to observe alterations of platelet aggregation; anti-I-CAM and anti-V-CAM antibodies were used to observe initial inflammatory responses, leading to pro-coagulative pathways. In addition, immuno-histochemistry results were compared to those of five unvaccinated subjects, deceased before April 2019, not exposed to the SARS-CoV-2 virus.

**Conclusions:** The epidemiological surveillance of the population has shown that vaccination programs have been successful in significantly reducing mortality and morbidity rates from SARS-CoV-2. The deaths occurring after the vaccine administration require a deep investigation in order to identify the mechanisms of death, the relation with vaccination, and the factors involved in possible individual susceptibility. These questions are of paramount importance to identify people with increased risk to develop severe adverse reactions, and hence to allow the patient’s safety in the context of global vaccination programs.

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### SARS-CoV-2, Vaccination, Immunohistochemistry

## H60 Postmortem Computed Tomography (PMCT) in Luxembourg: A Retrospective Analysis

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**Learning Overview:** After attending this presentation, attendees will have an overview of the use of PMCT in Luxembourg, including typical indications and strengths and weaknesses of the method.

**Impact Statement:** This presentation will affect the forensic science community by sensitizing forensic pathologists to the importance and benefit of PMCT by presenting typical applications, examination indications, and findings complementing autopsy results as well as selected case presentations.

PMCT was implemented in the routine work at the Institute of Legal Medicine in Luxembourg in January 2018. Since then, out of a total of 339 autopsies performed until July 31, 2021, 88 PMCTs (corresponding to 26 percent of autopsy cases) have been realized. Selection of cases to be scanned was based on the recommendations of the German Society of Legal Medicine (DGRM) on PMCT indications.<sup>1</sup> CT scans were evaluated by a clinical radiologist, and radiological diagnoses were compared with autopsy results.

The predominant indications for PMCT were traffic accidents (50 % of all PMCTs), followed by (suicidal or accidental) falls from great heights (20 %), and gunshot and stab wounds (11 % and 8 %, respectively). Less frequent reasons for a CT scan prior to autopsy were putrefaction, explosions, sudden death of infants or children, suspicion of medical malpractice, and homicidal strangulation.

Overall, a good agreement between radiological diagnosis and autopsy results was observed. As expected, CT was superior to autopsy, particularly in detecting gas or foreign bodies in vessels and tissues, and in detecting bony injuries (facial fractures, non-displaced cervical spine fractures, fractures of transverse processes of vertebral bodies, fractures of hand and foot skeleton, pelvic fractures). Classic autopsy, on the other hand, more reliably detected soft tissue, visceral organ, and vascular injuries (liver and spleen ruptures, aortic ruptures).

In conclusion, PMCT is a useful adjunct to conventional autopsy—particularly with regard to the detection of osseous lesions and foreign bodies. A CT indication according to Fischer et al. was established in about one-quarter of Luxembourg autopsy cases.<sup>1</sup>

The presented report clearly illustrates the benefit of PMCT prior to autopsy in certain cases.

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### PMCT, Forensic Radiology, Autopsy



## H61 Congenital Rib Abnormalities: A Presentation of Two Case Studies and a Literature Review

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the incidence and characterization of congenital rib abnormalities and their association with disease.

**Impact Statement:** This presentation will impact the forensic science community by presenting two case studies of congenital rib abnormalities and a review of the literature.

Congenital rib abnormalities are rare in the general population, with an estimated incidence of up to 0.6%, and are most often identified by radiographic imaging. They are generally categorized as bifurcated ribs with duplication of articulating ribs at the sternum, rib fusions resulting in a reduction of articulating ribs at the sternum, and rib foramen with a well-circumscribed round to oval intercostal space.<sup>1,2</sup> Bifurcated ribs (Luschka's bifurcated rib) are more common in males than females and occur most frequently in the third and fourth ribs on the right side.

Rib fusions most commonly involve ribs 1 and 2 and can be fused anteriorly or posteriorly.<sup>2,3</sup>

**Case 1:** A 59-year-old man with a history of substance use was found decomposing in his locked apartment with multiple empty methadone bottles at the scene. At autopsy, there was marked atherosclerosis of the left anterior descending coronary artery. The cause and manner of death is currently pending further investigation to rule out acute intoxication.

At autopsy, the left fifth rib was bifurcated at the anterolateral position, forming two distinct and separate sternocostal joints, with normal attachment and crowding on the left side of the sternum.

**Case 2:** A 3-month-old female infant was found unresponsive after bed-sharing with her mother. The infant was markedly below growth percentiles for age. It was not possible to determine the exact mechanism of death, and the cause of death was certified as Unexplained Sudden Death (Intrinsic and Extrinsic Factors identified). The manner of death was Undetermined.

At autopsy, the anterior halves of the bodies of right ribs 1 and 2 were fused, resulting in a single sternal end and costal cartilage for the fused ribs. The bone fusing the rib bodies was continuous and consistent in morphology. The contour of the ribs and their attachments to the spine were grossly normal.

Congenital rib abnormalities are rare and are likely underreported. A query of the Infant Injury Database (IID), a database containing soft tissue and skeletal findings from 868 infants and children ( $\leq 5$  years old) autopsied at the Harris County Institute of Forensic Sciences since 2010, identified only two cases using qualitative terms, including "fused," "joined," and "bifid."<sup>4</sup> Congenital rib abnormalities are generally considered benign anomalies but have been associated with Gorlin-Goltz syndrome along with multifocal basal-cell carcinoma of the skin, odontogenic keratocyst, facial abnormalities (frontal and temporoparietal bossing, hypertelorism, and mandibular prognathism), bilateral ovarian fibromas, and cardiac fibromas due to a mutation of the PTCH gene found on chromosome 9q in an autosomal dominant pattern.<sup>5</sup>

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### Congenital Rib Abnormalities, Luschka's Bifurcated Rib, Rib Fusion

## H62 A Forensic Investigation of Fatal Myocardial Infarctions—A Gender-Wise Comparison, Autopsy Findings, and Biological Rhythms: A Study From Greece

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**Learning Overview:** Attendees can expect to learn about gender-wise analysis of fatal myocardial infarctions that represent the most common cause of death in the developed countries.

**Impact Statement:** In Greece, a forensic practitioner is required by law to perform autopsies in many non-violent deaths, including many elderly deaths. Traditionally, autopsies provide reliable data for morbidity and mortality. This study sample can serve as the basis for further analysis to permit adaptation of more efficient preventive health policies.

Fatal Myocardial Infarctions (MI) represent a significant part of sudden-unexpected death cases referred for autopsy.<sup>1</sup> Gender-wise comparison of such cases, based on autopsies, has not previously been attempted in Greece. Demographic characteristics of the deceased, autopsy findings, and study of the biological rhythms may provide tools for better understanding of such cases.

Cases of MI-related deaths, examined during 2010–2017, were included in the study sample (827 cases in total). Medical history was obtained through hospital records, police information, and interviews with the relatives of the deceased.

Statistical analysis was performed using Stata/MP 13 (Stata Corp) and IBM® SPSS Statistics Version 20 (IBM). Statistical significance was defined as a 2-sided P value of <0.05. Pearson chi-square test and Student's *t*-test were appropriately used for comparison among categorical and continuous variables. The assessment of seasonal variation of fatal MIs was accomplished via two methods (chi square goodness of fit and Rayleigh test).

Regarding the social characteristics of cases, the mean age of males was lower than that of females (64.1 ±14.657 vs. 70.8 ±14.423 *p*<0.001). Marriage was encountered more frequently among males (58.5% vs. 37%, *p*<0.001). Females were more frequently widowed (32.6% vs. 7.6%, *p*<0.001); 30% of males vs. 14.9% of females were employed; *p*<0.001. 50.9% of males and 15.6% of females were retired.

Regarding the death conditions, females were more likely to die within their residence compared to males (53.6% vs. 42%, respectively; *p*=0.005). Furthermore, death inside a hospital was also more likely to occur among females (42% vs. 34.15%, respectively; *p*=0.049). On the other hand, a male predominance regarding deaths in public spaces was noted (24% vs. 4.4%, respectively; *p*<0.001). A fatal MI was more like to occur during physical activity among males (69.8% vs. 59.7%, respectively; *p*=0.010) and it appears that more females died while abed/sleeping (40.3% vs. 30.2%; *p*=0.010).

Symptoms prior to the event were reported more frequently among females (73.8% vs. 62.7%; *p*=0.024). No significant differences were observed as far as it concerns the mean duration of symptoms prior to the fatal MI between the two genders (Males: 3.86hrs [SD=8.556] vs. females: 3.29hrs [SD=5.519]; *p*=0.589).

The gender-wise comparison of cardiovascular risk factors (smoking, diabetes mellitus, dyslipidemia, family history of coronary artery disease, hypertension, alcohol consumption, history of prior MI) showed significant differences only for the following factors, which were more common among males: smoking (males 54.2% vs. females 35.3%; *p*<0.001), alcohol consumption (males 13.5% vs. females 6%; *p*<0.008) and history of prior MI (males 18.3% vs. females 7.2%; *p*=0.001).

Significant stenosis (>70%) in left anterior descending branch was more common among males (63.2% vs. 40.3%; *p*<0.001) while significant stenosis in the left circumflex or the right coronary artery appeared more often among females (44.1% vs. 28.7%; *p*<0.001) and (46.9% vs. 27.6%; *p*<0.001), respectively. Males suffered from significant three-vessel disease more often than females (33.3% vs. 17.1%; *p*<0.001). MI with Non-Obstructive Coronary Arteries (MINOCA) (defined as non-obstructive CAD<50% in all vessels) was more common among females (32% vs. 10.1%; *p*<0.001).

Myocardial scar evidence was more common among males (54.6% vs. 39.8%; *p*<0.001).

Monthly distribution of male cases appeared to be uniform using both chi-square goodness of fit and Rayleigh tests (*p*=0.204 and *p*=0.095). Regarding females, chi square goodness of fit demonstrated a non-uniform distribution of deaths (increase during April, decrease during June) in the case of monthly distribution (*p*=0.003); however, Rayleigh test did not yield the same result (*p*=0.758).

Gender-wise differences observed in the autopsy sample provide useful information that should be taken under consideration within the primary care settings. Cooperation between medical specialties is essential for the reevaluation of current preventive health policies.

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### Myocardial Infarction, Gender-Wise Comparison, Biological Rhythms

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### H63 The Determination of Manner of Death When Evaluating Intent: An Examination of Two Cases of Brodifacoum Adulteration of Synthetic Cannabinoids

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**Learning Overview:** This presentation examines autopsy findings in cases of Brodifacoum Adulteration of Synthetic Cannabinoids (BASC), discusses the manner of death, and examines how intent behind actions impacts the practice of forensic pathology.

**Impact Statement:** While several prior publications have focused on the clinical impact of Synthetic Cannabinoid-Associated Coagulopathy (SCAC), few have examined the forensic implications of this outbreak. This presentation reviews epidemiologic data of postmortem cases of SCAC, identifies autopsy findings of BASC in two cases within the Cook County Medical Examiner's Office (CCMEO), and explores the manner of death based on the circumstances of each case.

From May 2017 through October 2018, an outbreak of SCAC was recognized, with tests indicating BASC. These "superwarfarins" are warfarin derivatives commonly found in rodenticides. They have greater potency than warfarin and can cause internal hemorrhage and death.

National Medical Services (NMS) received 31 total cases of brodifacoum toxicity during this timeframe, including 7 cases additionally positive for other superwarfarins. NMS identified 19 positive samples from clinical entities and 12 positive postmortem samples. Clinical samples originated from referral labs, which may not reflect the actual patient locations. Clinical samples were sent from Illinois (5), Minnesota (5), Virginia (4), Utah (3), and Florida (2). Postmortem samples originated from Illinois (5), Wisconsin (5), and Maryland (2).

The 12 brodifacoum-positive postmortem cases ranged in age from 22–46 years, with a median and mean age of 33 years. Ten cases were male and 2 had no reported gender. Two cases from Illinois were positive for other superwarfarins. Ten of the 12 postmortem samples were tested for the presence of synthetic cannabinoids; 7 were positive for FUB-AMB and/or FUB-AKB-48.

The CCMEO autopsied two cases of BASC. In the first case, there was massive retroperitoneal hemorrhage without a definitive source of bleeding and no evidence of trauma. The cause of death was non-traumatic retroperitoneal hematoma due to brodifacoum toxicity with recent synthetic cannabinoid use. The manner was ruled "accident."

In the second case, autopsy findings included minor healing abrasions of the scalp with no underlying trauma to the skull or brain. Internally, a thin subarachnoid hemorrhage was present; sectioning demonstrated multiple foci of intraparenchymal and intravascular hemorrhage. This case was certified as complications of brodifacoum toxicity with recent synthetic cannabinoid use. The manner was ruled "undetermined."

The CCMEO policy is to certify most drug overdose deaths as accidents, as the decedent's intent was intoxication, not death. In some cases, clear suicidal intent with elevated drug levels can help determine the manner as suicide. In rare cases, the intent of the drug dealer may also influence the determination of manner.

Despite both cases involving young men self-administering adulterated synthetic cannabinoids, the circumstances surrounding the deaths were different. The first case was received at the beginning of the outbreak and was ruled "accident." There were no indications of suicidal intent and no implication of intentional poisoning and thus CCMEO policy was followed. The second case was received later in the outbreak, after multiple incidents were reported. The decedent suspected that he was intentionally sold BASC. While this information was relayed to the CCMEO investigators, it was not reported to the police and no arrests were made. Due to the suspicious circumstances, the manner of death was ruled undetermined as the allegation of the decedent could not be overlooked.

**General Statement of Conclusion:** To date, there is no explanation for the origins of the BASC and the intent behind the adulteration is unknown. Determination of manner of death is challenging when the circumstances surrounding the incident are not fully elucidated. In such cases, manner of death should be based on all available information, considering not only the actions, but the intent behind them as well.

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#### **Brodifacoum, Cannabinoids, Manner**

## H64 A Study of Post Severe Traumatic Brain Injury (sTBI): Pathological and Radiological Diffuse Axonal Injury (DAI) Changes

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**Learning Overview:** After attending this presentation, attendees will have a better understanding about the role of histopathology to ascertain the cause, survival time, and manner of death resulting from an intentional assault or accident. This is especially important where malicious head injury is suspected and the circumstantial data are contradictory or unknown by the pathologist.

**Impact Statement:** This presentation will impact the forensic science community by helping in ascertaining survival time and the degree of mechanical forces that developed during the traumatic event where malicious head injury is suspected, thereby evaluating the authenticity of statements made by the alleged perpetrator.

**Introduction:** DAI emerged as the most common and important pathological features of TBI and is considered as one of the main causes of unconsciousness in post-traumatic cases with the absence of detectable intracranial lesions on Computed Tomography (CT). This study assists in identifying the entire spectrum of brain injury, through pathological examination of the brain after postmortem.<sup>1-3</sup>

**Aims and Objectives:** to study post sTBI pathological and radiological DAI changes, and to compare the sTBI induced pathological and radiological DAI changes with non-TBI and control cases.

**Materials and Methods:** Sixty-four postmortem brain tissues from different sites (corpus callosum, thalamus, brain stem, and cortex) were collected from patients who died due to sTBI; 24 non-TBI samples of brain tissue and 12 control samples from individuals without any injury were also collected.

Patients whose Glasgow Coma Scale (GCS) score was  $\leq 8$  at the admission and whose age were above 18 years with positive CT findings were included in the study. Brain tissue was collected in 10% formaldehyde for pathological analysis after informed consent from the legally authorized representative of the patient.

The CT scan reports were analyzed for the presence of hematoma, contusion, fracture, and mass effect/mid line shift, which further classified according to Rotterdam CT classification.<sup>4</sup> CT images were analyzed from the Picture Archiving and Communication System (PACS) to calculate the DAI score.<sup>5</sup> After routine Hematoxylin-Eosin (H&E) staining and Immunohistochemistry (IHC) staining for  $\beta$ APP, microscopic changes were graded according to hypoxic changes, infarction, gliosis, axonal swellings, infarction, neovascularization, lipid laden macrophages, gemistocytic astrocytes, congestion/edema, hemorrhage, and vascular changes.<sup>6</sup> IHC of  $\beta$ APP was graded according to granular changes, axonal swellings, retraction bulbs, and presence of bands.<sup>7,8</sup>

**Results:** The average age of the patients is 39 years for the sTBI group, 33 years for the non-TBI group, and 36 years for the control group. Among the sTBI group, 61% of the patients have a GCS score of  $\leq 4$ . The male/female ratio (55/9) was in the sTBI group. The mode of injury was motor vehicle collision for 75.6% cases, followed by fall in 21.9%. According to collision type, rear impact crash was maximum with 28.6% followed by side impact crash of 22.5% in the sTBI group compared to the non-TBI group. In CT brain findings, hemorrhage is 79.3%, cerebral contusion is 55.9%, and subdural hematoma 49.1% was found to be significant.

Rotterdam CT score was found to be significant in the sTBI group compared to the non-TBI group with a maximum grade 2 score (i.e., 49.1%). DAI score was non-significant in the sTBI group compared to the non-TBI group with a maximum grade 1 score (i.e., 45.3%) followed by grade 3 in 34.0%.

The H&E and  $\beta$ -APP scores were found to be significant in the sTBI group compared to the non-TBI and control groups. According to the survival time of the patient, the H&E score is maximum median (min.-max.) 11 (9-13) and was found to be significant in  $>10$  days group compared to 0-6hrs, whereas  $\beta$ -APP scoring was able to identify axonal injury changes even at 0-6hrs following TBI.

**Conclusion:** DAI lesions are histopathological and neuroimaging hallmarks of DAI. CT imaging is quick, non-invasive, widely available, and has few contraindications, at the same time not sensitive to subtle DAI. Some patients with relatively normal CT scans may have significant unexplained neurological deficit. DAI changes were seen more in histology as compared to CT findings.  $\beta$ -APP serve as a gold standard for diagnosis of DAI. Forensic pathologists predominantly rely on gross findings to diagnose TBI; histological slides and paraffin blocks can serve as forensic evidence.

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### Traumatic Brain Injury, Diffuse Axonal Injury, Pathology

## H65 A Fatal Snap: Death by Extrinsic Percussion of the Neck

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**Learning Overview:** Given autopsy and histological data, this case highlights the importance of differential diagnosis between hypoxia from full-thickness tracheal rupture or fatal percussion of the cervical nerve bundle.

**Impact Statement:** This presentation will have a strong impact on the scientific community because it shows a unusual case of death by neck percussion, which is rare in the general population.

**Introduction:** Atypical neck compression is a form of asphyxia in which the fatal mechanism results from a combination of different pathophysiological mechanisms, such as respiratory, vascular, and nervous.<sup>1</sup> However, in cases where the impact on the neck is particularly severe, resulting in high kinetic energy transfer, death derives from percussion rather than compression of respiratory structures. Deaths from percussion are rare, being described mostly in professional athletes.<sup>2</sup> In addition, very violent blows can also cause significant injury to organs located in the neck loggias, such as tracheal total rupture.<sup>3-5</sup> In this work, a case of unusual death by extrinsic percussion of the neck is presented.

**Case Report:** A 43-year-old woman was on summer vacation with her boyfriend. As she passed by a garden closed to the public, she decided to take a photo. The woman clung with all her weight to a three-meter concrete beam suspended between two false columns, causing the structure to collapse. The beam broke in several places and fell on the victim, who was hit on the head, neck, and chest. Despite immediate resuscitation efforts, the woman died shortly after impact. Autopsy was requested by the judicial authority to clarify the cause of death. Externally, the woman exhibited typical signs of Morestin's ecchymotic mask, as well as rhinorrhagia, oral bleeding, bruising, and extensive ecchymotic-escharotic complexes. A characteristic excoriated ecchymotic complex in the form of a rail was observed on both the neck and chest, compatible with the profile of the concrete bar. Autopsy revealed several lesions, such as a subarachnoid hemorrhage, a mandible multifragmentary fracture, and amyelic cervical vertebral fractures. Key elements to establish the cause of death were full-thickness rupture of the trachea and esophagus, as well as significant hemorrhagic infiltration of the right cervical neurovascular bundle. These autopsy findings, together with the absence of typical vital signs of tracheal injury, such as blood in the airways or cervical subcutaneous emphysema, led pathologists to conclude that death was immediately caused by atypical ab extrinsic percussion of the neck. Stimulation of reflexogenic areas, such as sensory branches of the superior laryngeal nerve or carotid sinus, determined the onset of cardiac inhibitory phenomena, which, together with asphyxia mechanisms caused by compression on the neck, brought about sudden death. These conclusions were further supported by histological examination, which showed significant hemorrhagic infiltration of the right neurovascular bundle and the absence of pulmonary intra alveolar hemorrhage. This report presents an unusual case of death from cranio-cervical-thoracic polytrauma, with several major injuries, such as full-thickness rupture of the trachea, and minor ones, such as hemorrhagic infiltration of the cervical nerve bundle.

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### Polytrauma, Ab Extrinsic Neck Percussion, Asphyxia

### H66 A Case of Traumatic Internal Carotid Artery Dissection: A Rare Event With Devastating Consequences

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**Learning Overview:** This report is about a case of Traumatic Internal Carotid Artery Dissection (TICAD), a condition usually diagnosed when cerebral damage has already occurred. Thus, even if it is rare, a prompt diagnosis is essential. This case highlights the importance of the adoption of a TICAD screening strategy in cases of blunt traumas, especially when a motor vehicle is involved.

**Impact Statement:** This presentation will have a great impact on the forensic sciences community because it demonstrates the need to establish screening guidelines for the diagnosis of TICAD, which unfortunately are not yet in place.

**Introduction:** Internal Carotid Artery Dissection (ICAD) occurs when a false lumen is created by the blood penetration into the arterial wall because of internal elastic lamina discontinuation. About 20% of cerebral infarctions in the young adult population are due to ICAD.<sup>1,2</sup> Both extracranial and intracranial carotid arteries could be involved, and the pathophysiological mechanism could be spontaneous (SICAD) or traumatic (TICAD). Carotid injuries could complicate the 0.32% of cases of general blunt trauma, and it seems that the percentage increases in severe multiple traumas. When TICAD occurs, a direct or indirect cervical injury is usually described and is often related to motor vehicle accidents.<sup>3-5</sup> Unfortunately, it is frequently diagnosed when neurological symptoms have already occurred leading to permanent neurological impairment or even death.<sup>6,7</sup> In blunt carotid injury, morbidity is estimated at up to 80% and mortality up to 40%.<sup>7,8</sup> Specific TICAD screening guidelines are missing. Nevertheless, TICAD should be taken into consideration in the case of a young adult or middle-aged patient involved in severe blunt trauma. Herein, a rare case of TICAD is presented.

**Case Report:** A 54-year-old man with an unknown medical history was involved in a car accident while driving a car, manifesting a transient sudden loss of consciousness shortly after. The first medical evaluation revealed acceptable vital parameters, frontal skin abrasion, and foot crush injuries with exposed fracture. An X-ray examination also showed two vertebral fractures (one at the right arch of C1 vertebra and the other at D10 vertebra's body) and multiple left pulmonary contusions. No ischemic or hemorrhagic brain injuries were present and surgery for the foot fracture was planned. Two days later, the neurological situation changed as the patient could not move the left upper limb, and a brain Computed Tomography (CT) scan plus CT angiography revealed a right posterior cortico-subcortical temporo-parietal insular ischemic lesion with median shift caused by a right ICAD with almost completed lumen obstruction. A revascularization procedure was not indicated, and the physical examination showed left hemiplegia, right-sided head deviation, right eye divergent strabismus, bilateral reactive miosis, Cheyne-Stokes respiration, and drowsiness. The day after, a brain CT scan showed ischaemic lesion progression. A decompressive hemicraniectomy and a partial temporal lobectomy were performed, but the neurological status deteriorated further until brain-death was declared. The forensic autopsy revealed humble adventitial hemorrhagic infiltration of the right ICA slightly below the carotid bifurcation. The right common, internal, and external carotid arteries were sampled, and the histological examination confirmed the presence of intramural hematoma with intimal and media laceration, and a thrombus into the lumen.

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#### Internal Carotid Artery, Dissection, Trauma

### H67 The Hazard of Grabbing a Quick Bite: A Case of Fatal Choking on Ham

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**Learning Overview:** Although fatal food asphyxia is not so unusual, it is relatively uncommon among healthy adults. This case highlights the need to not exclude the hypothesis of fatal food asphyxia in lower-risk individuals and, at the same time, the importance of ruling out all of the possible alternative causes of death.

**Impact Statement:** This presentation will impact the forensic science community by showing an unusual case of fatal asphyxia that occurred in a man with no risk factors for food inhalation. In such cases, the forensic pathologist plays a key role in establishing the real cause of death.

**Introduction:** Fatal asphyxiation by a foreign body is not as rare as expected. According to the National Safety Council of the United States, foreign body inhalation represents the fourth-leading cause of accidental death.<sup>1</sup> Several studies demonstrated that food represents the most common type of foreign body implicated.<sup>2</sup> Fatal choking predominantly occurs in infants and small children because of their oral exploration behavior and uncoordinated swallowing reflex.<sup>3,4</sup> Conversely, it is relatively unusual in adults, due to the larger diameter of the airways, and mostly involves fragile people such as elderly or neurologically impaired people.<sup>5,6</sup> In this report, a case of a healthy middle-aged man who choked on 30g of unintentionally inhaled ham is presented.

**Case Report:** A 42-year-old man was eating his meal during lunchtime when he suddenly manifested dyspnea and dry heaves. His colleagues did not understand what was happening until he feebly asked for help since he was choking. They immediately called the emergency service and performed the Heimlich maneuver. The emergency personnel found the patient cyanotic and in cardiac arrest; despite rescue attempts, he died. A necropsy investigation aimed at establishing the cause of death and whether earlier health treatment would have saved the man was performed. External examination showed a single petechial hemorrhage in the conjunctiva and hematic staining in the oral cavity. Internal examination revealed the presence of 30g of sliced ham that completely occluded the airways from laryngeal aditus to the tracheal bifurcation. The bolus was molded to the shape of the obstructed airways. Lungs showed subpleural petechiae. At the histological investigation, signs of acute pulmonary emphysema were found. Autopsy excluded any concomitant disease and toxicological analysis was negative. There was no sign of trauma. Witnesses' description of death circumstances corroborated the hypothesis of mechanical asphyxia. For these reasons, it was concluded that this was an infrequent case of fatal food asphyxia in a healthy adult due to accidental food aspiration. It was possible because of the special characteristics of the food bolus. In fact, some food items pose a greater choking hazard due to their size, shape, or texture, becoming dangerous also for lower risk individuals. The investigation, also based on the perused documentation, allowed the exclusion of the health care professionals' liability.

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#### Foreign-Body Asphyxia, Choking on Food, Bolus Deaths



## H68 Massive Insulin Injection: A Rare Case of Suicide

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**Learning Overview:** Suicide by insulin represents a challenging diagnosis for forensic pathologists; therefore, a high level of suspicion needs to be maintained for clues that insulin may be involved, requiring complete autopsy investigation and a toxicological and histological analysis with correlation to circumstantial evidence.

**Impact Statement:** This presentation will impact the forensic science community because of the rarity of this manner of death, in consideration of the difficult determination of insulin in biological samples, especially in the postmortem setting.

This presentation aims to discuss a rare case of suicide by massive insulin injection. Suicidal insulin overdose is an unusual cause of death, often undervalued due to non-specific autopsy findings.

Analysis of insulin overdose is a challenging task in forensic pathology because of the complexities in toxicological analysis. It is therefore essential to prove the role of such a substance as the cause of death by performing a thorough toxicological, histological, and immunohistochemical analysis. Accidental fatal insulin overdose is the most common manner of death, followed by suicide, and very rarely homicide.

Insulin is a hormone that regulates glucose blood concentration. Proinsulin molecules consist of two chains along with a connecting peptide (C-peptide) before secretion. Exogenous insulin administration is able to suppress secretion of C-peptide and to diagnose hypoglycemia induced by exogenous insulin, it is necessary to test serum C-peptide concentration. Insulin administration should be meticulously managed since it could lead to severe hypoglycemia, coma, and even death. The mechanism of death linked to insulin overdose is hypoglycemic encephalopathy, which can damage irreversibly the central nervous system. The normal glucose concentration of blood is 3.9–7.1 mmol/L; when the blood glucose concentration is lower than 2.2 mmol/L, the glucose concentration in brain tissues is close to zero. Several studies show that myocardium is not sensitive to hypoglycemia and can normally work under such conditions, thus explaining why there are no evident pathologic alterations in the hearts of deaths by insulin overdose.

Postmortem analysis of insulin is challenging because of the rapid occurrence of decompositional changes and blood hemolysis after death. It is impossible to determine whether hypoglycemia was the cause of death based on postmortem glucose levels. Such difficulties emphasize the pivotal role of crime scene investigation in the evaluation of insulin-related deaths.

This report presents the unusual case of a 55-year-old Caucasian female found dead at home. At the crime scene investigation, there were no signs of forced entry into the victim's apartment and no signs of violence were found on the deceased body. The victim was found lying on the bed, fully dressed, and close to her feet, three empty insulin pens were found. A further pen, not used, was found after moving the dead body. Six handwritten letters were found, confirming the suicidal purposes of the woman.

During the autopsy, no macroscopic pathological findings were found in the major organs. Blood, humor vitreous, urine, bile samples, and major parenchymal organs were taken for toxicological and histological analysis, respectively. Histological analysis was inconsistent, except that neuronal degeneration was conspicuous in the cerebral cortex and basal ganglia of the brain. Toxicological analyses were negative for drug abuse and alcohol intake, thus revealing the presence of acetone, the simplest and smallest ketone body, insulin metabolites.

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### Insulin, Suicide, Acute Intoxication

## H69 When Chronic Venous Insufficiency Becomes a Life-Threatening Condition: A Case of Fatal Hemorrhage From a Varicose Veins' Ulcer

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**Learning Overview:** This case is unusual because the cause of the blood loss was not immediately evident at the crime scene, whereas the huge amount of blood all over the place may induce suspicion of a violent crime. In such cases, the skin ulcer may be very tiny. Therefore, the forensic pathologist needs to carefully look for it while evaluating the body. The aim of this presentation is to emphasize the role of a complete and accurate corpse inspection and to provide a complete overview of the needed examinations.

**Impact Statement:** This presentation will impact the forensic science community since it illustrates a possible event that each pathologist may encounter in his/her career. Attending this presentation, attendees will have improved their knowledge about such suspicious forensic scenarios.

Chronic Venous Insufficiency (CVI) of the lower limbs is a common condition in Western countries.<sup>1</sup> The pathogenesis of CVI and associated Varicose Veins (VVs) is related to valve reflux and incompetence.<sup>2</sup> Possible complications of these conditions are skin ulceration and ulcer rupture. Ulcer rupture could be spontaneous or a consequence of mild/trivial traumas.<sup>3</sup> In such cases, a large amount of blood around the victim may lead to the hypothesis of violent death, whereas the real cause of death usually is hemorrhagic shock due to the spontaneous bleeding from the ulcer. Therefore, the forensic pathologist has to distinguish between natural or violent death, which is not always obvious. Even if blood loss is a frequent scenario, the diagnosis of hemorrhagic shock is not easy to prove, and often is a diagnosis of exclusion.<sup>4-6</sup> Herein, the case of a woman found dead in a pool of blood with no apparent traumatic lesions is presented.

**Case Report:** A 90-year-old woman, who lived alone, was found dead at her home. The corpse was slumped on the toilet bowl with a huge blood stain on the floor. A closer look at the body revealed a stain of blood running down her left leg, as well as a blood-soaked towel tied around it. There were many bloody footprints in the bathroom and other rooms. A judicial autopsy was performed to establish the cause of death and exclude the hypothesis of a crime. The external examination showed pale skin and mucous membranes, abundant blood staining of the lower limbs with a point-like solution of continuity, with telangiectasias on the nearby skin, located on the mid-lateral aspect of the left leg, 34cm above the calcaneus. A careful squeezing of the lesion resulted in leaking of some blood drops. There were no other traumatic lesions. The internal examination revealed pale organs. Histologic studies were undertaken on formalin-fixed tissue from all organs, showing non-specific morphological findings (hemorrhage and parenchymal necrosis) consistent with "shock organs." The left leg skin lesion was also sampled. The histological examination showed a segment with removal of the epidermis and dermis with consequent deep dermis exposure. Additionally, diffuse chronic atrophic skin changes were seen. Toxicological screening was negative. The complete autopsic procedure and histological examination of organ tissues excluded any alternative cause of death, so eventually the death was attributed to hemorrhagic shock.

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### Varicose Veins, Ulceration, Fatal Hemorrhage

## H70 Death by Arterial Injury: Two Unusual Cases of Sharp Force Fatalities

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**Learning Overview:** In the reported cases, a great number of peculiarities were observed: unusual location of the injuries, armpit in the first case and lower leg in the second; manner of injuries, accidental fall in Case 1 and stabbing in Case 2; cause of death, massive blood loss owing to a single artery injury in both cases.

**Impact Statement:** This presentation will impact the scientific community by showing how only a complex investigative approach, with correct interpretation of circumstances of death, in conjunction with a detailed autopsy examination (both macroscopic and microscopic) along with toxicological analysis, represents the best practice for the discrimination of such unusual deaths.

Fatalities due to sharp force trauma may be homicidal, self-inflicted, or accidental, and they represent 10-20% of clinical forensic examinations. Moreover, homicide by sharp force (stabbing) is one of the most common in European countries, involving generally domestic or interpersonal conflict. Wounds to the head, limbs, hands, neck, or back are consistent with homicide, while injuries affecting the anterior part of the trunk, the neck, or forearms, are predictive of suicide.

This presentation aims to discuss two rare cases of sharp force fatalities, showing how forensic pathology methodology, including death scene investigation, autopsy examination, and toxicological analyses, are pivotal to detect the manner of death. These two rare cases deal with an accidental event and a murder.

The first is about a complete transection of the axillary artery caused by an accidental stab injury on the right armpit of a 57-year-old man. The event dynamics relate to a fall on a piece of sharp glass. Toxicological analysis was extremely meaningful, as it proved the presence of alcohol in blood (2.56g/L), urine (3.55g/L), and gastric (2.48g/L) fluid samples.

The second case deals with another atypical death of a 41-year-old man: a single stab wound on the leg causing a 0.5cm linear full-thickness lesion of the anterior tibial artery. The cause of death was attributed to massive external blood loss owing to the right anterior tibial artery lesion, following a scissor stabbing.

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### Sharp Force Injuries, Arterial Lesion, Accidental Death

### H71 Postmortem Degradation of Porcine Skeletal Muscle Proteins During a Heat Wave in Northern Germany

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**Learning Overview:** With this presentation about postmortem protein degradation in different outdoor conditions and research regarding postmortem protein decomposition for Postmortem Interval (PMI) estimation, attendees will gain information about an innovative method to determine the time since death—a crucial topic in forensics.

**Impact Statement:** A precise determination of the time since death is of high importance in forensic routine. Further research in this field is required in order to expand the prevailing range of applications in forensic PMI estimation. Especially, analysis of muscle protein degradation has shown its potential to contribute to according mid- and long-term delimitations in practice. This publication will improve our understanding on postmortem protein degradation in realistic outdoor conditions in particular.

A most precise determination of the PMI is one of the major tasks and a continuous challenge in forensic routine. An innovative approach—based on the biochemical analysis of protein degradation patterns—has been developed that contributes to expand the prevailing range of applications in forensic PMI estimation. In particular, specific time points of degradation events of certain proteins provide reasonable markers for PMI delimitation. Nevertheless, considerable research is yet required to increase our understanding of protein decomposition and how it is affected by individual and environmental factors in order to provide a broad applicability of this method.

Following the scientific method, it is first required to exclude as many influencing factors as possible—for example by investigating postmortem protein degradation under controlled (and) standardized conditions. Previous studies/experiments showed predictable results of protein degradations patterns at different ambient temperature settings in a controlled environment. In a next step, it is important to apply the method in natural outdoor conditions in order to investigate the robustness of this method toward certain environmental influences.

Therefore, a field study was conducted using five pig carcasses, left to decompose under natural environmental conditions until full skeletonization. Muscle samples of the M. biceps femoris were collected daily, processed via Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and degradation patterns of selected proteins were identified by Western blotting. Unexpectedly, the planned sampling period turned out to be in the middle of a heat wave with temperatures significantly beyond average, allowing unique insights into degradation events under these (increasingly frequent) circumstances.

Due to the high ambient temperatures and substantial insect activity, the cadavers decomposed within only few days, allowing sufficient tissue sampling only until day 7. Results of the protein analysis interestingly showed that some proteins remained stable over the sampling period, while others displayed complete degradation of the native form, in part accompanied by distinct degradation products at defined PMI phases. This study provides evidence that muscle protein degradation patterns compare well between results from standardized experiments and this field study, despite extreme conditions.

Together with data from previous experiments at different ambient temperatures, results are expected to provide additional data to establish a reliable model of postmortem protein degradation for time since death estimation.

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#### Protein Degradation, PMI, Environment

## H72 A Cocaine-Related Left Ventricle Rupture: A Case Report

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**Learning Overview:** The goal of this paper is to examine the contribution of cocaine abuse in a rare case of heart rupture.

**Impact Statement:** This presentation will impact the forensic science community through the provision of a proper autopsy technique, a complete toxicological analysis, and a histological examination, in order to describe the correlation between the role of acute consumption of cocaine and cardiac rupture in cocaine abuse.

Cocaine, an alkaloid extracted from the leaves of coca, is a sympathomimetic drug that mainly determines effects on the central nervous system and cardiovascular tone. Cardiovascular complications are among the most common and dangerous adverse effects of cocaine abuse and include cardiac arrhythmias, hypertension, acute myocardial infarction, infective endocarditis, ventricular dysfunction, dilated cardiomyopathy, hypotensive shock, and stroke.<sup>1</sup> This presentation describes a cardiac death of a cocaine chronic abuser. An accurate autopsy, macroscopic, microscopic findings, and toxicological analysis confirmed that cocaine had a crucial role in the cause of death. The habitual use of cocaine is likely to be the main feature of the histological heart changes. The acute intake of cocaine induced an increase of circulating catecholamines, with a consequent increase of inotropism and chronotropism ending in a massive ventricular rupture.

**Case Report:** A 56-year-old man was found dead on the street in the early morning. The body temperature was 100.4°F (38.5°C), the environmental temperature was 60.8°F (16.5°C), hypostasis moved completely to the newly dependent zones, and rigor mortis was absent. At external examination, no lesions were found. A complete autopsy was performed. Anatomical dissection by single planes was performed.<sup>2</sup> The heart postmortem examination showed a pericardium blood collection of 510ml and a full thickness tissue lesion of 3.5 x 3cm at the posterior wall of the left ventricle, which exposed the ventricular cavity and the papillary muscles. The histological investigation of heart samples showed diffuse areas of myocardial scarring, contraction band necrosis, perivascular fibrosis, colliquative myocytolysis, myofiber breakup, severe coronary sclerosis with critical stenosis of the anterior descending coronary artery, with up to 95% occlusion of the vascular lumen.<sup>3</sup> Consequently, to verify the presence of xenobiotic substances, toxicological investigations were performed. The ratio (brain/blood) between the concentration of cocaine found in the brain (772.3ng/ml) and in blood (364.4ng/ml) was approximately 2.1, and affirmed that there was a recent consumption (15 minutes) of cocaine. The concentrations of cocaine detected (ng/ml), in the samples examined, are: urine 1,007.4, gastric contents 2.183, lung 261.4, liver 673.3, and kidney 721.4. The concentrations of BEG detected (ng/ml), in the samples examined, are: blood 140.8, urine 111.3, gastric contents 111.6, brain 95.9, lung 626.7, liver 637.3, and kidney 164.2. A hair sample was analyzed, too, and allowed to assess the chronic abuse of cocaine (cocaine concentration 38.7ng/ml, BEG concentration 4.7ng/ml). Conclusions: cocaine increases systemic blood pressure, heart rate, and contractility—the major determinants of myocardial oxygen demand. Concurrently, cocaine induces coronary vasoconstriction, thereby limiting oxygen delivery to the heart. This vasoconstriction occurs both in normal and diseased segments of artery but its effects are more pronounced in diseased segments. This vasoconstriction results from alpha-adrenergic stimulation coupled with endothelial endothelin (vasoconstrictor) production and nitric oxide (vasodilator) reduction.

This case serves as a reminder that in cases of heart rupture, a meticulous postmortem examination (proper autopsy technique, a complete toxicological analysis, and histological examination) and a careful history search for substance abuse is fundamental, in order to achieve a correct postmortem diagnosis.

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### Cocaine, Acute Intoxication, Heart Rupture

### H73 Ischemic Heart Disease Due to Intramyocardial Amyloidosis and Undiagnosed Plasma Cell Myeloma

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**Learning Overview:** This presentation will inform attendees that occlusion of intramural small vessels by amyloid in the setting of systemic amyloidosis is a known, but infrequently encountered, cause of ischemic heart disease that should be considered in decedents with a clinical history strongly suggestive of ischemic heart disease but without epicardial coronary artery disease or known risk factors.

**Impact Statement:** Awareness of this entity will broaden the differential diagnosis in these rare but challenging cases of apparent ischemic heart disease, leading to correct identification and certification of cause of death, as well as providing timely answers for families and treating clinicians.

Greater than 90% of cases of ischemic heart disease, including angina pectoris and myocardial infarction, are attributed to obstruction of the epicardial coronary arteries by atherosclerotic lesions, which often progress slowly over time in patients with known risk factors that include obesity, hypertension, elevated cholesterol, smoking, diabetes, and family history positive for myocardial ischemia.<sup>1</sup> In rare cases, however, ischemic heart disease can occur in individuals without clinically apparent coronary artery disease or known risk factors.

Described here is a case of sudden death in a 61-year-old man with a 15-month history of exertional chest pain, diminished exercise tolerance, and fatigue. He was an avid runner, a non-smoker, and had no history of hypertension, hyperlipidemia, diabetes, or known family history of heart disease. Cardiac workup eight months prior to his death included an **Electrocardiogram** (EKG) showing atrial fibrillation with rapid ventricular response and incomplete left bundle branch block, echocardiogram showing a minimally diminished left ventricular ejection fraction that resolved on conversion to normal sinus rhythm, and left heart catheterization showing large, widely patent coronary arteries. He was heard moaning in the night and was subsequently discovered unresponsive by his family. Autopsy confirmed the absence of significant atherosclerosis in the major coronary arteries. However, the myocardium showed a mottled appearance, and multiple foci of acute and subacute myocardial infarction were identified on microscopic examination. Diffuse amyloidosis with luminal narrowing was identified in the intramural small vessels, highlighted by Congo Red special stains. Diffuse vascular amyloidosis was also identified in the lungs, liver, kidneys, spleen, thyroid, small bowel, and skeletal muscle. Finally, there was diffuse infiltration of the bone marrow by atypical, CD138-positive plasma cells. The cause of death was thus listed as ischemic heart disease due to amyloidosis of intramyocardial arteries, due to plasma cell myeloma.

Occlusion of intramural small vessels by amyloid in the setting of systemic amyloidosis is a known, but infrequently encountered, phenomenon. Furthermore, it is difficult to diagnose in the clinical setting and is most often discovered at autopsy or explantation.<sup>2</sup> Intramyocardial small vessel amyloidosis should be considered in decedents with a clinical history strongly suggestive of ischemic heart disease but without epicardial coronary artery disease or known risk factors.

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#### Ischemia, Coronary, Amyloidosis

## H74 An Indoor Postmortem Dog Scavenging: A Case Study

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**Learning Overview:** After attending this presentation, attendees will be able to focus the importance of external examination, of autopsy findings, and of the effects of contribution of small and big fauna to postmortem change and artifacts.

**Impact Statement:** During this kind of crime scene investigation, it is important to accurately discriminate the presence/absence of self-defense injuries on the deceased body, the amount of blood present in the scene, the presence of paw prints deriving from animal's paws, accessibility to the pets/wild animals, and between antemortem and postmortem wounds.

The discovery of decomposed bodies in a domestic setting is not an uncommon occurrence. Many animal species are members of this food chain. However, postmortem animal scavenging of their owner is not commonly reported but can occur when the main predisposing factors are social isolation, pets living free in the house, and a medical condition causing sudden death.<sup>1</sup> Postmortem animal activity is an important step in incorporating protein, fat, and carbohydrates in corpses to the food chain. Outdoor corpses may be attacked by many predacious and scavenger animals and exposed to complete destruction, and the bones and belongings of the dead person may be scattered all over a large area due to postmortem animal activity. Indoor corpses may be attacked by pets, domestic dogs, cats, ants, and rodents during the postmortem period.<sup>2</sup> Frequently, the face, hands, and legs are destroyed by postmortem animal interference as they are unclothed and thus easily accessible.<sup>3</sup>

**Case History:** A 76-year-old male was found dead in his house in an advanced state of decomposition. The house was in poor hygienic condition, and there was garbage and feces of probable canine origin everywhere on the floor. No blood was found at the scene. He was lying supine on the kitchen floor: the neck and head were exposed, while the chest and the lower part of the body were covered with a gym suit. The external examination showed a general mummification of tissues coexisting with an almost complete skeletonization of the viscerocranium with a complete absence of the teeth, tongue, and upper airways. The eyeballs and nasal cartilages were absent. Despite the skeletonization of the viscerocranium, the ears were only partly removed, and the scalp of the neurocranium had an increased consistency and hair. During the site inspection, the officers found a small dog, likely responsible for the scavenging. The next day a Computed Tomography (CT) scan evaluation was performed, which excluded traumatic head and bone lesions. A complete autopsy was performed one week after the discovery. While the brain was completely absent, the heart, lungs, and abdominal organs were reduced as the state of putrefaction advances with a massive and diffuse larval infestation. According to the postmortem changes, the time between the death and the recovery of the body was about 45–60 days. Histological examination of the wound edges on the face showed no obvious vital reaction. Toxicological analysis of the blood showed no significant alcohol levels or any other toxic substances.

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### Postmortem Damage, Predators, Skeletalization

### **H75 A Multidisciplinary Investigation of a Corpse Found at the Sea, With Particular Focus on the Use of Barnacles for the Estimation of the Time Spent in Water**

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**Learning Overview:** The goal of this paper is to present the multidisciplinary investigation performed on a unknown corpse found in water. The corpse was in advanced state of decomposition and the study of barnacles led to the estimation of the Postmortem Interval (PMI).

**Impact Statement:** This presentation will impact the forensic community by informing attendees of the importance of a multidisciplinary forensic approach and the use of barnacles for PMI estimation.

Human remains can be found in water environments for multiple reasons such as accident, natural or mass disasters, homicide, and suicide. The characteristics of aquatic environments are many and varied and all unique in terms of biological, chemical, and physical properties. The decomposition process and the colonization of the remains are strictly related to these characteristics. As any other forensic medical investigation, when a corpse is recovered from an aquatic environment, primary tasks are the confirmation of the personal identification, the determination of the cause of death, and the estimation of the time since death (PMI).

To date, considering the difficulties in the evaluation of postmortem changes, several studies have suggested the use of aquatic organisms, such as macroinvertebrates and algae in both freshwater and saltwater environments, to aid in the estimation of the PMI. In particular, for cases that occurred in seawater, the analyses of the barnacles (Crustacea: Cirripedia) colonizing the remains and the clothes and the objects associated with it have proven to be able to provide important information both on the provenance of the corpse and the PMI. The present casework reports the multidisciplinary investigation performed on a corpse recovered in the Strait of Sicily in the Mediterranean Sea. The corpse was found in an advanced stage of decay, partially skeletonized and dismembered, with diffuse adipocere. Goose barnacles (*Lepas anatifera* L.; Crustacea: Cirripedia: Pedunculata) were found colonizing the items connected with the corpse (a watch, a single sock, and a shoe), the teeth, the exposed bones, and, for the first time, were also observed attached directly on the corpse's skin. A complete medicolegal investigation comprehensive consisting of a Computed Tomography (CT) scan and diatom-test allowed ascertainment of the cause of death by drowning, while the use of barnacles and oceanographical data were used to estimate the floating time and the journey traveled by the corpse. The DNA profile of the subject was uploaded in the official international databank; however, at present the identity of the subject remains unknown. Despite this case being still open, the multidisciplinary approach to this complex investigation should be used as a guideline for future similar cases.

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#### **Postmortem Investigation, PMI, Barnacles**



## H76 Bodies in a Pool of Blood: Murder or Natural Death?

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**Learning Overview:** The aim of this presentation is to prove that an accurate crime scene investigation, external examination, and autopsy is essential in case of sudden death due to the rupture of varicose veins.

**Impact Statement:** The purpose of this presentation is to consider, in crime scene investigations characterized by large bloodstains, the rupture of venous varices as the cause of death. Therefore, it is crucial to perform a detailed external examination of the lower limbs and a complete autopsy.

Varicose veins are tortuous and pathologically dilated vessels, due to the chronic increase in intraluminal pressure and the weakening of the supporting vessel wall.<sup>1</sup> Venous reflux originates from a connective tissue disorder or from the failure of the venous valves, which results in retrograde flow and stasis of venous blood in terminal branches of veins.

Some of the typical symptoms of chronic venous insufficiency include aching, itching, swelling, and heaviness in the legs, which can progress to skin changes and ulceration. There may be lower limb bleeding if a very superficial and friable vein is involved.<sup>2</sup>

**Case Report:** This presentation describes three cases of sudden death due to the rupture of varicose veins. In Case 1, a 61-year-old overweight male was found at home; the crime scene was characterized by some large bloodstains on the floor, rooms, and corridor, with some shoe print marks. The corpse presented a blue color of both lower limbs, related to a venous insufficiency. In the left foot, at 0.39 inches (1cm) inferiorly from the lateral malleolus, there was a bleeding round-shaped skin laceration, with a diameter of 0.11 inches (0.3cm). Case 2 was a 48-year-old male with congenital malformations in the chest, found in his room by his parents, surrounded by an extended blood area. The corpse presented an accentuation of the subcutaneous venous district that appeared purple with tortuous course and a cutaneous bleeding laceration on the extreme distal of left leg. Case 3 was an 83-year-old female undertreated with oral anticoagulants, found once again in a pool of blood. The external examination showed an irregular-shaped skin laceration measuring 0.27x0.59 inches (0.7x1.5cm) in the lateral side of the right leg. In Cases 1 and 2, a complete lower limb dissection was performed; malleolar regions showed: dorsal metatarsal veins, dorsal cutaneous venous arch, lateral marginal vein, small saphenous vein, and lateral dorsal cutaneous nerve. In Case 1, the muscular heads were detached from the distal insertion and overturned to allow the isolation and study of the vascular-nervous component (anterior artery and anterior tibial vein, a superficial peroneal nerve, and deep peroneal nerve). In Case 2, a hydraulic test was executed in the blood vessel below the skin lesion of the left leg; after clamping the interested vein tract, a syringe of water was administered into the vein, and liquid loss was observed from the blood vessel.

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### Rupture of Varicose Veins, Sudden Death, Large Bloodstains

### H77 An Unusual Suicide Using a Screwdriver: A Case Report

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**Learning Overview:** After attending this presentation, attendees will be aware that the presence of multiple weapons at the scene necessitate careful examination of the wounds' entrances, tracks, and exits.

**Impact Statement:** The impact of this presentation is that screwdrivers, although non-lethal most of the time, can be used as a method of suicide.

Suicide by sharp force to the neck is relatively uncommon, and complex suicide using more than one method is rare. Screwdrivers are unusual weapons for suicide. Herein is described a case of a middle-aged male with history of depression, valvular heart disease, and methadone use, who was found face down in a pool of blood in his locked apartment. At scene were a blade knife with dried blood and a bottle of soap spilled on the floor. In the kitchen, open boxes of naloxone were seen, along with a white powder. There were three empty methadone bottles found on the bed. There were suicide notes found on the bed and in the kitchen. The decedent had a sharp force stab wound on the right side of the neck that penetrated up to the strap muscles. The wound on the midline of the neck had a screwdriver embedded in the wound track with a hilt mark. This stab wound had sharp margin and angled ends, consistent with a stab injury from a knife. The lower margin of the wound showed some irregularity of the sharp margin, which probably represents an additional injury created by the screwdriver. The stab wound(s) penetrated the anterior neck, the tongue, and came out into the oral cavity and penetrated the palate of the upper jaw with the tip of the screwdriver embedded within the roof of the hard palate. The tongue showed three stab wound tracks with a star shape (screwdriver) wound on the right-side angle of the tongue, and two teardrop wounds (knife) on the right anterior and posterior tongue. There were corresponding wounds on the pharynx in the midline of the upper jaw (corresponding to the star-shaped screwdriver), and teardrop wounds on the right side of the pharynx. The track wounds stopped at the roof of the upper hard palate and did not penetrate the skull cavity or injure the brain. Postmortem toxicology showed methadone (1447ng/ml) and fentanyl (0.24ng/ml). Death was attributed to combined methadone intoxication and exsanguination of blood from the neck and tongue, caused by the knife and the screwdriver stab wounds, with preexisting heart disease and fentanyl intoxication as a contributing factor. This study describes the first case of combined knife and screwdriver stab wounds using the same point of entry and resulting in multiple paths.

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**Suicide, Multiple Stab Wound, Screwdriver**

## **H78 Post-Vaccination Myocarditis and Thrombosis: Monitoring Strategies and Impact on the Vaccines' Benefit-Risk Assessment**

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**Learning Overview:** This presentation will inform attendees of the impact of mass vaccination during the COVID-19 pandemic and its adverse effects.

**Impact Statement:** This presentation will impact attendees by presenting what is known of the autoptoc and histological findings concerning post-vaccination death.

**Introduction:** The COVID-19 pandemic has made it necessary to shorten the time of research because of its high transmissivity. Two types of vaccines were developed with two different technologies: viral vector and messenger RNA (mRNA). Thrombosis and myocarditis are considered the most frightening complications of these vaccines.<sup>1-4</sup>

**Materials:** Case 1: A 45-year-old female with abdominal pain was hospitalized 12 days after the administration of the first dose of viral vector vaccine. The Computed Tomography (CT) scan revealed a splenic-mesenterial-portal thrombosis and the laboratory tests highlighted a severe thrombocytopenia without predisposing factor. Case 2: A 61-year-old female suffering from hepatic cirrhosis and renal failure was found dead the morning after the second dose of mRNA vaccine; it was a case of sudden death because of the absence of detectable signs or reported symptoms. Case 3: A 63-year-old male, suffering from diabetes mellitus, hypertension, bronchopulmonary chronic obstructive disease, and schizophrenia was found dead less than three days after the first dose of mRNA vaccine.

**Results:** Case 1: Autoptoc and histologic findings confirmed a mesenteric, hepatic, pancreatic, and splenic venous thrombosis. Case 2: The autopsy and the histologic exams only revealed the presence of acute pulmonary edema. Case 3: The autopsy and the histologic exams showed pulmonary thromboembolism, aortic thrombosis, and myocarditis.

**Discussion:** The post-vaccination myocarditis and thrombosis currently remain the most frightening adverse effects observed during the mass anti-COVID-19 vaccination. It is already possible to outline a few differences between the two complications, although the final epidemiological-statistical results are still waited upon completing the vaccination campaign. Myocarditis was observed most frequently following the administration of mRNA vaccines' second dose, as opposed to thrombosis mainly occurring following the first dose of viral-vector vaccines, less often following mRNA vaccines, as the Case 3 illustrates. Myocarditis further involved more male subjects younger than 30 years, although it can also afflict elderly subjects, such as in Case 3; thrombosis had a female prevalence and a high age heterogeneity. The median time to onset of symptoms was three days for myocarditis, and 8-16 days (viral vector vaccine) or <3 days (mRNA vaccines) for thrombosis. It is assumed that the mRNA could be considered as antigen by the immunological system activating an innate and acquired immune response with a consequent proinflammatory cascade developing the myocarditis.

There would seem to be an immune-mediated mechanism at the base of thrombosis, with antibodies against the complex PF4-polyanions as protagonists. A genetic predisposition is hypothesized in both cases. Myocarditis showed a basically positive outcome in contrast to the high mortality of thrombosis; for this reason, the diagnosis and therapy of myocarditis can be made at the moment of hospitalization and modulated according to the specific case. The thrombosis' poor prognosis requires an early diagnosis and specific monitoring strategies put in place between the third and tenth day following the vaccination through the monitoring of platelets and antibodies.

The benefit-risk assessment for both types of vaccines demonstrated a positive balance, independent of age or gender, because of the positive outcome of myocarditis and the low thrombosis' incidence (approximately 1:100.00/1.000.000). The results of autoptoc exams were awaited to evaluate vaccine-related mortality and exclude the cases in which vaccination was only the context of the event, such as in Case 2.

**Conclusion:** Further studies and final epidemiological data are awaited to verify the positive balance of the benefit-risk assessment for both types of COVID-19 vaccine, the incidence and mortality rate of post-vaccination cardiomyopathy and thrombosis, and their long-term impact. The current lot suggests that monitoring and prevention strategies for the thrombosis could be useful during the mass vaccination for COVID-19 with both types of vaccines.

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### Vaccine Adverse Effects, COVID-19 Vaccination, Vaccine-Related Complications

## H79 Sudden Cardiac Death and Thymic Hypertrophy: A Recurrent Cycle in Medicine

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**Learning Overview:** After attending this presentation, attendees will appreciate the importance of the extensive heart examination in sudden unexpected deaths and will also have a short review concerning the “myth” of thymic death.

**Impact Statement:** This presentation will encourage further in-depth studies concerning the relation between thymic hypertrophy and cardiovascular pathology by the forensic science community.

Sudden cardiac death is a major public health issue worldwide. The exact incidence rate of sudden cardiac death in young adults remains unclear and is generally thought to be underestimated. The most common mechanism of a cardiac arrest leading to sudden death is abrupt ventricular fibrillation because of an underlying cardiovascular disease.<sup>1</sup>

This presentation reports the case of a 24-year-old man, with a silent personal and familiar medical history, who suddenly collapsed in a restaurant during dinner with workmates. The man did not smoke and was an amateur soccer player; he was 172cm tall and weighed 78kg.

Toxicological examinations were negative. Immunological examines excluded anaphylactic shock. The autopsy did not show macroscopic lesions, but the extensive study of the heart’s conduction system along with specific histological examinations detected atrioventricular node fibrosis. The fibrosis has been pointed out using Masson’s Trichrome stain.

Furthermore, the autopsy drew attention to the 13cm wide hypertrophic thymus. The histological examinations of the thymus did not relieve any structural alteration. In the past, thymic hypertrophy was considered a cause of sudden death and part of some nosographic entities like status lymphaticus or thymic asthma.<sup>2,3</sup> The so-called “thymic death” remained consigned to the history books until 2017 when a Chinese study<sup>4</sup> reclaimed the hypothesis of the correlation between thymic hypertrophy and sudden death through an immunological point of view.<sup>4</sup>

Sudden unexpected death remains a big issue for pathologists and requires full autopsy and ancillary exams to be carried out. The present case reported infrequent heart disease, with any known underlying cause, and thymic hypertrophy. Is this a coincidence? Should the myth surrounding the “thymic death” be re-examined?

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### Sudden Cardiac Death, Atrioventricular Node Fibrosis, Thymic Death

### **H80 A Rare Case of Sudden Death Due to Endomyocardial Fibrosis in Italy: A Differential Diagnosis With Other Causes of Restrictive Cardiomyopathy**

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**Learning Overview:** This presentation allows attendees to acquire more information about endomyocardial fibrosis, especially regarding its macroscopical and histological characteristics.

**Impact Statement:** Attendees will be impacted by being informed that in sudden death, a detailed and complete differential diagnosis could be very important, including any uncommon pathology.

Endomyocardial Fibrosis (EMF) is an uncommon restrictive cardiomyopathy leading to congestive heart failure. The disease is characterized by deposition of fibrous tissue in the endocardium and, to a lesser extent, in the myocardium, usually affecting the apical region of the right or left ventricle, or both. It is most common in regions with high air humidity within tropical countries, in particular, in the eastern and central regions of Africa. Rare cases were native Europeans who stayed in tropical countries for a long period of time. Endomyocardial fibrosis frequency has a bimodal peak at age 10 and age 30. The majority of studies do not show any difference between the sexes.

In this case, a 45-year-old Indonesian woman was admitted to an Italian hospital with nausea, vomiting, abdominal pain, and tachyarrhythmia. Atrial fibrillation was found at Electrocardiogram (ECG) and blood tests showed mild hepatic function alterations. Thoraco-abdominal echography and Computed Tomography (CT) showed bilateral pleural effusions, ascites, and hepatomegaly. Physical conditions and laboratory results worsened rapidly, followed by multi-organ failure. Systolic and diastolic functions of the left ventricle were found to be strongly compromised at US. Death occurred 28 hours after admission.

An autopsy was performed to clarify the cause of death. It showed jaundiced skin, bilateral pleural and pericardial effusions, ascites, mild cardiomegaly, ventricular endocardial fibrosis, a thrombus in tight junction to the left ventricular wall, and hepatic necrosis.

Histological investigations revealed a massive endomyocardial fibrosis, easily detected through Hematoxylin-Eosin, Verhoef-Van-Gieson, Azan-Mallory stain. Congo Red stain was also performed and was negative. Toxicological investigations were negative; the paracetamol level was under the therapeutic range. Pneumococcal, EBV, CMV, HBV, HCV, and HIV antigens and bacterial cultures on blood samples were all negative.

The cause of death was a global cardiac dysfunction caused by a restrictive cardiomyopathy in an Indonesian woman affected by an undiagnosed and asymptomatic endomyocardial fibrosis. In particular, differential diagnosis between other causes of restrictive cardiomyopathy (amyloidosis, endocardial fibroelastosis) have been excluded.

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#### **Endomyocardial Fibrosis, Autopsy, Sudden Death**

## H81 Formalin-Fixed Paraffin-Embedded (FFPE) Extraction of DNA From Tumor Samples: A Revolutionary Breakthrough

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**Learning Overview:** The goal of this presentation is to inform attendees as to how important DNA extraction from formalin-fixed material will be for forensic science.

**Impact Statement:** Previous studies have been performed on FFPE DNA extraction; however, those samples were specifically non-tumor samples. This presentation looks at the efficacy of this extraction method of tumor samples. This is important for forensics since tumors are a part of normal tissue. This study is to compare tumor tissue to normal tissue.

Extraction of formalin fixed tissues would be an extraordinary advancement in DNA for the forensic science community. An extremely beneficial impact it creates is FFPE extraction, which can recover vital DNA that was previously unrecoverable, such as cases where tissue was previously scarce and degrading and needed fixation.<sup>1,2</sup> Recovery is invaluable for these unknown or extremely uncommon DNA samples.

There have been previous techniques on non-tumor specimens, such as prostate biopsies, liver, brain, and other autopsy samples.<sup>3</sup> These studies led to the question of FFPE DNA extraction from tumor blocks originating from differing organ samples to determine the efficacy of said technique. So far, DNA extraction is entirely possible; however, further testing is still needed to determine the best methods and techniques for a complete, successful extraction.

In this presentation, the FFPE QIAGEN<sup>®</sup> tissue kit was used for extraction of the DNA, and results were taken from multiple tumor blocks with sources from colon and kidney samples taken from both St. Rita's and Lima Memorial hospitals in Lima, OH.<sup>4</sup> As of present, Reverse Transcription Polymerase Chain Reaction (RT-PCR) was used to determine positive or negative extractions as well as sex to look for contamination between different gendered blocks of the same tumor. The results showed a positive DNA sample for both the male and female extractions with 17.5985nm/μL and 376.194nm/μL of DNA extracted, respectively.<sup>5</sup> Further testing and extractions are still needed to determine efficacy, yet the current data yields promising results for future developments.

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### FFPE, DNA, Extraction

## H82 Chronic Mineral Oil Aspiration: A Case of Exogenous Lipoid Pneumonia

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**Learning Overview:** After attending this presentation, attendees will be able to identify peculiar macroscopic and histopathologic findings associated with lipoid pneumonia.

**Impact Statement:** This presentation will impact the forensic science community by improving the ability to identify a rare form of interstitial pneumonia, combining anamnestic and clinical data with histological findings.

Lipoid pneumonia is an unusual form of interstitial pneumonia characterized by the presence of lipid drops in macrophages and throughout the pulmonary parenchyma. Depending on the source of the lipid/oil in the respiratory tract, its etiology can be endogenous (bronchial obstructions such as those caused by neoplasms) or exogenous (aspiration of animal fats or vegetable/mineral oils). Due to its rarity and its insidious clinical presentation, characterized by non-specific respiratory symptoms and radiological findings, the diagnosis of lipoid pneumonia may be challenging in both clinical and postmortem settings.<sup>1,2</sup>

**Case Report:** An 83-year-old man, living in a care facility since 2012, with an history of cognitive impairment, Parkinsonism, dysphagia, and diabetes mellitus developed symptoms of a lower airways infection in November 2020. Therefore, an antibiotic (cephalosporin) was added to the man's usual medications, which consisted of an oral anticoagulant, antidiabetic drugs, a neuroleptic sedative, and a mineral oil as a laxative. Despite the given therapy, his symptoms progressively worsened over the following weeks; for this reason, different antimicrobial agents were administered (fluoroquinolones) and the patient tested negative twice for SARS-CoV-2. On the last days of December, signs of pulmonary edema and cardiac failure were detected; all therapeutic efforts were unsuccessful, and the death was declared a few hours later.

Postmortem examination revealed pleural and pericardial effusion, right-heart dilation, bullous pulmonary emphysema, yellowish mucus in the tracheobronchial tree, pulmonary edema, and parenchymal consolidation of the lower lobes. All the collected organ samples were formalin fixed and paraffin embedded, then stained with Hematoxylin-Eosin (H&E). In the site of the pulmonary consolidation, histological findings showed large fibrotic areas interspersed with cystic spaces surrounded by macrophages containing globules of lipid material, dilated capillaries, and fibroblasts. In addition, an organizing exudate composed of numerous granulocytes, plasma cells, and lymphocytes was observed.

The macroscopic and histologic postmortem findings allowed identification of the cause of death as a respiratory failure resulting from acute on chronic pulmonary alterations, which could be attributed to lipoid pneumonia. The etiology of this condition may be ascribed to the continuous aspiration, due to the man's dysphagia, of small droplets of mineral oil, chronically taken for laxative purposes.<sup>3</sup>

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### Lipoid Pneumonia, Mineral Oil, Lung Disease



### H83 Tell Me What You Ate and I'll Tell You When You Died

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**Learning Overview:** This presentation aims to highlight the importance of tanatochronology.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence on the importance of the gastric content study in order to establish the time of death and the role of different eating habits.

Assessing the correct timing of death is one of the biggest priorities in forensics, being of great interest when determining and identifying an eventual liability in someone's death. It is made harder when bodies are kept at a controlled temperature in cold rooms. The study of gastric content is, hence, crucial.

The following work will analyze and discuss the case of a 76-year-old male, found dead on the morning of November 27, 2017, at a residence in southern Italy by his son at 9.30 a.m. Although noticing the total absence of vital signs, the son called the emergency number. The ambulance arrived at 12:40 p.m., after the intervention of the police. The son eventually made a statement against the emergency department, stating that an immediate intervention at the time of the first call would have saved his father. No investigation on the spot was performed. In order to replay a judicial question, it was essential to identify the time of death, so the autopsy was performed.

The external cadaveric examination showed fixed hypostases, consensual with the supine lying, resolution of the corpse rigidity, and body temperature lower than the ambient one, compatible with the permanence of the corpse in the cold room. Autopsy revealed bilateral pulmonary congestion, heart concentric increase, and dilated aortic lumen with atheromatous alterations, in particular at the level of the aortic ascending tract. The cause of death was identified in the fissuring of the vascular intima of the ascending aorta with consequent onset of arterial thromboembolic phenomena. The stomach was filled with abundant poltaceous material with corpuscular elements, including fragments of mollusks and vegetables in the absence of appreciable free liquid content. Toxicological analyses excluded the presence of xenobiotic substances involved in death.

In this case, not being able to base the thanatochronological evaluation on classic cadaveric phenomena, the study of the gastric contents and its emptying was helpful. The gastric emptying variables are therefore correlated to quantity and quality of food eaten.<sup>1-3</sup> Generally, abundant solid meals leave the stomach after the liquid component, and higher calorie meals leave the stomach slower. Easily digestible solid meals leave the stomach between three and five hours, while abundant solid meals leave between five and eight hours.

In the present case, the gastric content was abundant without free liquid content. So, in consideration of the quantity and complexity of the gastric contents and of scientific data concerning gastric physiology, it was possible to conclude that the death occurred within 5–8 hours from the end of the meal. Moreover, the presence of fragments similar to mollusks and vegetables led to the belief that the gastric content was compatible with that deriving from a lunch or more likely a dinner, according to the most common eating habits in the birthplace of the deceased (Naples), of his residence, and the discovery of the body.

In any case, even in the hypothesis that the death occurred very late with respect to the interval indicated above (up to about 5.30), a timely intervention by the 118 health workers following the first phone call, the deceased would have been dead at least four hours anyway. As a result, not only studying the gastric content crucial to determine the time of death in absence of other elements but also knowing the cultural differences among eating habits and geographical areas made it possible to refer a meal to a certain time of the day.

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#### Tanatochronology, Gastric Content, Eating Habits

## H84 Sometimes Common Stuff Can Kill: Pay Attention to Your Phone

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**Learning Overview:** After attending this presentation, attendees will be aware of an unusual kind of electrocution caused by low-voltage electric current and of the hazardousness of smart phone use in bathtubs.

**Impact Statement:** This presentation will impact the forensic science community by explaining the importance of an integrative and multidisciplinary approach in cases of death by suspected electrocution.

Death from electrocution is caused by the passage of electric current through the body. It can cause a wide range of injuries, local or systemic, or even death, depending on several specific characteristics, such as amperage, voltage, and frequency. Every electrical device, under certain circumstances, can cause electrocution, even an apparently harmless mobile phone. Although for most people picking up the phone anytime is as natural as breathing and it is regarded as one of the most run-of-the-mill, low-risk things we can do, actually it is not always true.<sup>1,2</sup>

In a small city in northern Italy, a 32-year-old man was found dead in his bathtub after an electrical blackout. The emergency response team could only confirm the death. The body was naked and presented an intense rigor mortis. Furthermore, the man's mobile phone, still plugged to the main electricity circuit via a power cord and an extension cord, was found immersed in the water of the bathtub. A forensic investigation was requested by the prosecutor in order to clarify the cause and manner of death. A site inspection and a forensic autopsy were performed, during which tissue samples and biological fluid were taken in order to carry out histological and toxicological investigation. Sections of skin lesions, collected with a ceramic scalpel, were analyzed using suitable histological staining (Hematoxylin-Eosin [H&E] and Perls' staining), then Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-Ray Spectroscopy (EDS).<sup>3,4</sup> Moreover, an engineer was asked for additional consulting in order to better define the dynamic of the event. At external examination, circumscribed and superficial burns with a central zone of pallor were detected; they involved the right arm, the right hand, and the trunk. Here they were characterized by a striped-shaped lesion, extended from the right chest to the ipsilateral inguinal area, ending in a figured, rectangular burn with a size of 20 x 8cm (this lesion matched well with the phone found in the bathtub). At autopsy, the morphological findings were non-specific. Several petechiae were detected on conjunctiva, the oral cavity, heart, and lungs, together with diffuse visceral congestion. The histological examination of the skin lesions revealed the typical microscopic picture of a current mark. The diagnosis of electric mark was supported by Perls' staining and by the SEM analyses, which revealed metallic particles on the skin sample. Toxicological analysis conducted on the blood was negative. Furthermore, the engineering investigation found that the phone charger was counterfeit and did not meet safety regulation standards. In particular, the isolation was not sufficient to prevent an electric shock. On these bases, it was possible to conclude that the cause of death was an accidental electrocution.

To correctly identify this type of death, the integrated use of different tools is recommended, such as macroscopic observation, H&E, and iron-specific staining, and SEM analysis. Most importantly, every electronic device found on the scene should be considered as a potentially fatal source of electricity and properly investigated, asking for engineering consulting.

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### Electrocution, SEM, Accidental Death

### H85 Drowning at Home: Risk Factors and Prevention in Adults

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**Learning Overview:** Attendees of this case series presentation will learn about the risk factors, autopsy signs, and prevention strategies of at-home drownings.

**Impact Statement:** This case series will impact the forensic community by showing that certain risk factors in adults are associated with at-home drownings, exploring autopsy findings in drownings, and suggesting prevention strategies for vulnerable patient populations.

Drowning occurs when submersion of the nose and mouth in water causes respiratory impairment. Since the volume of water required for drowning only needs to be enough to submerge the nose and mouth, there are multiple ways to drown at home. When filled with water, bathtubs, sinks, toilets, and buckets all pose potential risks. Drowning prevention is most commonly discussed with pediatric populations, but adults with certain medical and psychiatric conditions also have an increased risk of drowning. The risk of drowning increases when a patient is incapacitated in some way, whether through intoxication, seizure, syncope, cardiac arrhythmia, psychiatric illness, or other methods.<sup>1,2</sup> The identification of risk factors and patterns in drownings can inform prevention strategies.

This presentation will examine five cases of drownings. The decedents range in age from 22 to 76 years old, and all are found deceased at home. The locations of these drownings include two bathtubs, a toilet, a pan, and a kitchen sink. The contributing causes include drug use, epilepsy, cardiovascular disease, schizophrenia, and Parkinson's disease. This presentation will explore the multiple risk factors for drowning, describe the autopsy findings associated with drowning, and discuss prevention strategies related to these cases.

One of the goals of forensic pathology is to identify patterns in mortality to further knowledge and to aid public health measures for prevention. Autopsy findings that indicate drowning, but are not necessarily universally present, include foam in the airway, lung hyperinflation, increased lung weight, and watery stomach contents.<sup>3</sup> Since the autopsy findings for drowning are non-specific, careful examination of the scene is important for accurate determination of the cause of death. There are multiple risk factors for drowning, including drug use, seizures, cardiac conditions, and psychiatric conditions, and patients with these factors should be educated on prevention strategies.<sup>1,2</sup> Strategies to prevent at-home drownings can include using showers instead of bathtubs, not leaving uncovered collections of water at home, and preventing vulnerable states of unconsciousness. By examining the patterns of at-home drownings, recommendations for prevention can be targeted to at-risk patient populations.

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#### Drowning, Home Safety, Prevention

### H86 The Differential Diagnosis Between Accidental Injury and Fatal “Shaken Elderly Syndrome”: Insights From a Case Report

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**Learning Overview:** After attending this presentation, attendees will be aware that intracranial hemorrhages in elderly people may suggest fatal physical abuse.

**Impact Statement:** This presentation will impact the forensic science community by contributing to highlight the importance of a multidisciplinary approach to make differential diagnosis between accidental craniocerebral injuries and abusive head trauma in the elderly. This will increase awareness of medicolegal implications of the clearly underestimated elder abuse phenomenon.

An 82-year-old woman was found at home unconscious and unresponsive to the application of painful stimuli. Her nephew called an ambulance, reporting she had accidentally fallen, so the woman was taken to the emergency department. As multiple contusive lesions were present, on suspicion of physical abuse, the judicial authority was informed. A Computed Tomography (CT) scan identified bilateral subdural hematoma and left subcortical cerebral hemorrhage. No indication of neurosurgical treatment was given. Four days after unsuccessful intensive care, brain death was pronounced.

The autopsy performed three days later showed the presence of multiple excoriations and polychromatic bruises associated with macroscopic hematic infiltrate, mostly distributed among the head, thorax, arms, and legs. It showed a single hematoma of the inner aspect of the scalp, no fracture of the skull, bone callus on anterior arch of four ribs, and a mesenteric-ileal hemorrhagic area. After formalin brain fixation, the presence of bilateral subdural hemorrhage of about two centimeters (maximum thickness) and left temporo-occipital subcortical cerebral hemorrhage was confirmed. Basic histological examination using hematoxylin-eosin stain showed heterogeneous chronology of cutaneous lesions (from 4-5 days to 14-16 days before death) and different components of subdural hematoma (one dating 4-5 days before death, the other dating 16-18 days before death). Histological examination of the other organs was non-contributory.

Cause of death was classified as brain dysfunction due to large subdural hemorrhage. Since no coagulative disorders as well as spontaneous intracranial hemorrhages (such as intracerebral hemorrhage) were present, a traumatic origin of subdural hematoma was identified. According to the lack of signs of direct head trauma and the chronological correspondence between chronic subdural hemorrhage and the arms' bruises, subdural hemorrhage was considered the result of bridging veins rupture due to brain acceleration in the skull secondary to violent shaking.<sup>1</sup>

“Shaken Baby Syndrome” is extensively described in literature, but intracranial findings of characteristic abusive head trauma are not limited to pediatric patients. When shaking as a traumatic mechanism is used in elderly people, it can be hard to differentiate abusive intracranial findings from spontaneous ones.<sup>2,3</sup> Further studies are needed to identify effective and appropriate diagnostic criteria for “Shaken Elderly Syndrome,” particularly focusing on differences between spontaneous subdural hemorrhages and those due to shaking.

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#### Shaken Elderly Syndrome, Subdural Hematoma, Elder Abuse

### H87 A Fatal Case of Delayed Post-Tonsillectomy Hemorrhage: Potential Medicolegal Implications

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**Learning Overview:** After attending this presentation, attendees will have a better knowledge of the frequency and significance of post-tonsillectomy complications, their outcomes, and potential medicolegal implications.

**Impact Statement:** This presentation will impact the forensic science community by presenting a peculiar case report in which the autopsy findings and the clinical evidence helped clarify the possible medicolegal issues involved in a fatal delayed Post-Tonsillectomy Hemorrhage (PTH) in a pediatric patient.

Tonsillectomy is one of the most common surgical procedures worldwide. Although it is considered routine surgery, several complications may happen. Among these, PTH is the most common and significant complication. The vast majority of PTH is generally easily managed, but life-threatening bleeding may rarely occur.

According to the literature, PTH represents a significant portion of malpractice claims against surgeons. Potential technical errors during the surgery represent the most common complaint. However, in cases with unintentional outcomes, questions arise about the surgical indication. Both surgical mistakes and the absence of a clear indication for surgery are considered medical errors, and medical malpractice can occur.

Presented here is the case of a 2-year-old Caucasian child with a history of severe Obstructive Sleep Apnea (OSA). A rhinolaryngoscopy examination revealed grade IV tonsillar hypertrophy. A sleep study revealed numerous, severe episodes of obstructive apnea with moderate/severe desaturations. The clinical scenario was consistent with severe OSA, and tonsillectomy was indicated.

Surgery was performed without intra-operative or early post-operative complications. The patient was discharged after 24 hours of uneventful observation in the hospital. A follow-up visit was performed six days after the surgery and did not reveal any complications or abnormalities. Approximately 36 hours after the follow-up visit, the patient experienced profuse hematemesis and loss of consciousness. He was immediately transferred to the local emergency department by ambulance, but he died during the travel. A medical malpractice lawsuit was filed against the hospital, primarily against the surgeon and the attending physician who performed the follow-up visit after the tonsillectomy. Thus, the legal authority ordered a medicolegal autopsy.

The autopsy revealed features of wound healing grossly in the tonsillar bed bilaterally, with evidence of fresh hemorrhage in the left tonsillar bed where an absorbable stitch was properly located. Dissection of the pharynx revealed possible involvement of the ascending palatine artery, in the context of healing process and artifacts due to the recent surgery. The lungs showed focal areas of possible blood aspiration. The digestive tract was filled with dark blood starting from the stomach and extending up to 160cm within the small bowel. Grossly normal, clear bowel content was present in the terminal ileum and colon. No other abnormalities were detected.

The forensic expert was asked to comment about potential medical errors and malpractice. A multidisciplinary approach was required to answer the questions asked by the legal authority. The approximate time of bleeding was roughly estimated based on the autopsy finding and a review of the clinical medical literature. Moreover, a thorough evaluation of the clinical history, documents, and guidelines allowed comment about the treatment's appropriateness and indication to the surgery.

A thorough discussion of the case from pathological, medicolegal, and clinical points of view will be presented to the attendees.

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#### Forensic Science, Tonsillectomy, Medicolegal Implications

### H88 Disseminated Fungal Infections at Forensic Autopsy

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the types of situations where disseminated fungal infections may be evident at autopsy, as well as the pathologic findings in such cases.

**Impact Statement:** This presentation will impact the forensic science community by highlighting several cases where disseminated fungal infections were found in cases presenting for medicolegal death investigation.

Fungal infections are an increasing source of morbidity and mortality worldwide with multiple factors contributing to their rise. One critical factor is a growing number of immunocompromised or transiently neutropenic patients due to hematopoietic stem cell and solid organ transplants, as well as the rising use of immune-modifying therapies.<sup>1,2</sup> Climate change and our growing population are influencing the prevalence of endemic fungi and human exposure to them. Diagnostic tools for identifying fungal disease have rapidly advanced, including genetic testing through Polymerase Chain Reaction (PCR) and other molecular techniques.<sup>1</sup> However, symptoms of fungal infection are often non-specific and may mimic other infections or other disease, so correct diagnosis may be delayed and only occur after other work-up has failed.<sup>3</sup> To diagnose and treat fungal infection requires awareness of fungal disease features. The public health threat of fungal infection is best addressed by increasing understanding and recognition of these diseases and risk factors.<sup>3</sup> To this end, this study reports three cases of death due to fungal infections occurring in a Midwest United States medicolegal death investigation office.

In the first case, a 22-year-old White female, with a history of Noonan syndrome and a seizure disorder present from childhood, was discovered deceased in her home without any history of acute preceding illness. At autopsy, she was discovered to have evidence of chronic disseminated histoplasmosis, with granulomatous inflammation affecting her lungs, lymph nodes, spleen, kidney, and liver. The disseminated fungal infection was considered incidental.

The second case concerns a 24-year-old Cambodian male with a recent history of being “extremely ill,” but who had reportedly declined medical care. He had a history of marked weight loss and night sweats. He was reported to have a history of possible tuberculosis; however, according to records, his cultures never grew. He experienced a fatal cardiorespiratory arrest at his home. At autopsy, microscopic examination of the lungs showed diffuse granulomatous inflammation along with mixed inflammatory cell infiltrate with special stains positive for fungal organisms. There was also granulomatous inflammation in the lymph nodes, spleen, kidney, liver, pancreas, and thymus. Additionally, his skin showed fungal ulcerations. He was determined to have diffuse, systemic blastomycosis, which was considered the Cause Of Death (COD).

The final case involves a 59-year-old White female with a history of immunosuppression due to a bilateral lung transplant for the treatment of idiopathic pulmonary fibrosis. She was also suffering from chronic transplant rejection and was awaiting second transplant. She was found deceased in her home from apparent massive hemoptysis due to pulmonary hemorrhage from invasive aspergillosis with associated vascular wall necrosis.

Fungal diseases may have a diversity of presentation, from relatively asymptomatic to fatal, and disease can occur in a wide range of patients. These cases may be difficult to correctly diagnose or differentiate from other infectious agents; hence, the absence of diagnosis in these patients prior to their deaths.<sup>4</sup> These examples demonstrate an opportunity for improvement in treatment of fungal disease, through increased awareness and suspicion of fungal infection, especially with changing environmental conditions, worldwide human migration, and increasing immunosuppressed patients. Pathologists can be instrumental in diagnosing fungal infections, providing important feedback to clinicians when such cases are not identified during life.

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#### Fungal, Infection, Mycoses

## H89 Krönlein Shots and Pseudo-Krönlein Shots

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**Learning Overview:** After attending this presentation, attendees will be familiar with the forensic pathology eponym term “Krönlein shot” as it pertains to certain firearm-related injuries. In addition, attendees will be introduced to a new term, “pseudo-Krönlein shot,” which refers to non-firearm traumatic brain injuries which simulate true Krönlein shots.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new term, “pseudo-Krönlein shot,” to describe expulsion injuries of brain hemispheres occurring in non-firearm trauma situations.

The terms “Krönlein’s shot” and “Krönlein shot” are eponyms used in forensic pathology to describe the expulsion of an intact brain hemisphere (or both hemispheres) in high-velocity firearm injuries of the head.<sup>1-3</sup> The phenomenon is named for the famous German surgeon Rudolf Ulrich Krönlein, who first described brain evisceration from a military rifle wound in 1899.<sup>1-3</sup> Krönlein shots typically occur with high-energy contact-range gunshot wounds of the head, such as occur with shotguns or high-velocity rifles. This study presents several classic cases of Krönlein shots, then presents a series of non-firearms related traumatic deaths that demonstrate cerebral hemisphere expulsion injuries similar to what occurs with Krönlein shots. The moniker “pseudo-Krönlein shot” is suggested for such injuries.

Pseudo-Krönlein shots may occur in a variety of traumatic situations, including those involving blunt force/deceleration/crushing injuries, sharp force/chop injuries, and explosion injuries. Examples of each of these scenarios will be presented. Blunt force/deceleration/crushing injury cases include motor vehicle collisions, a skydiving incident, and a crane accident. An example of sharp force/chop injury involves an airplane propeller. An example of an explosion injury involves an incident involving a tire blowing off of the rim of heavy construction machinery.

Although described as being “rare” by some authors, firearm-related Krönlein shots are encountered relatively frequently in busy medical examiners offices in geographic regions where firearms are common. Such injuries are especially common in contact wounds of the head using shotguns or high-velocity rifles; however, similar shots can occur with contact wounds using high-energy handguns, as well as non-contact wounds involving high energy weapons, such as high-velocity rifles or shotgun slugs. Expulsion injuries of entire cerebral hemispheres, or substantial portions thereof, are classic findings, with brain segments typically being found relatively close to the body. Pseudo-Krönlein shots demonstrate similar expulsion injuries of the brain; however, the brain segments may be a considerable distance from the body, compared to those produced by firearms, depending on the underlying forces responsible for the injuries.

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### Krönlein Shot, Firearms, Mimics

## H90 Mutual Homicide Including Immediate Revenge Homicide by Two Unrelated Assailants Utilizing One Sword

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**Learning Overview:** After attending this presentation, attendees will review and become familiar with external and internal wounds consistent with mutual homicide (fatal stabbing with immediate revenge homicide) with a single weapon utilized by two assailants.

**Impact Statement:** Recognition of mutual homicide with one weapon can conserve police investigation resources; characteristics of injuries can support police inquiry with autopsy evidence. Difficulties with transporting a decedent with the sharp force object in place should be overcome in order to permit optimal evaluation of the second decedent.

Deaths of two unrelated persons after mutual combat, utilizing the same sharp force weapon, are rare. This presentation describes mutual homicide by two unrelated decedents, utilizing one weapon; the death of the second person may be described as an immediate revenge homicide.

Emergency medical services were called for two severely injured victims in the same mobile home community. The unrelated decedents lived on adjacent lots and had a history of arguments. On the day of death, the 66-year-old man, who had acquired a 39½" (100cm) "samurai sword," challenged the 19-year-old man; the older man cut the younger man on the leg before stabbing him in the chest through the ribs, liver, right hemidiaphragm, and right lung. Witnesses said the combatants were briefly scuffling on the ground. Autopsy evidence (cuts on the volar surfaces of fingers of both hands) supported the contention that the younger man pulled the blade out of his body himself. Before dying, he impaled the older man with the sword, through the chest wall, right lung, heart, esophagus, descending aorta, left lung, and left eighth rib. Both combatants became unresponsive. Death ensued for the older man before Emergency Medical Services (EMS) arrival, and for the younger man shortly after arrival in the emergency department.

At autopsy, the younger man showed limited emergent medical intervention, including endotracheal intubation and intravenous and intraosseous catheterization. A postmortem chest X-ray showed profound leftward tracheal shift with right hemopneumothorax. Autopsy documented the stab wound and extremity cuts as described. Natural pathology was limited to a benign follicular adenoma of the thyroid gland. Postmortem toxicology was negative for drugs or alcohol.

The older man presented with the "samurai sword" in place, wrapped in a second body bag. 28" (~70cm) of hilt and blade protruded at an angle from his chest; slightly more than 11" (28cm) remained in the body; a cardboard protector beneath the body prevented the protruding tip from enlarging its 3/8" (~1cm) partial exit. He had no other injuries. X-rays demonstrated the weapon clearly. Autopsy demonstrated the wound path, along with gastric pouch blood, hemothorax, hemopericardium, and visceral pallor. Natural pathology included hypertensive and atherosclerotic cardiovascular disease, emphysema with an unsuspected right lung malignancy, and cirrhosis of the liver with steatosis. Postmortem toxicology was negative for drugs; the blood alcohol was 0.159% by weight by volume.

Autopsy findings assisted the police in corroborating the story and determining that no further investigation was required into the deaths of either the older man who initiated the attack or the younger man who succeeded in an immediate revenge homicide. The Commonwealth attorney declined prosecution. Both cases were closed on completion of the police and autopsy investigation.

Multiple homicides occurring in one event are often domestic. "Mutual combat" has been utilized as an alternative description for domestic violence, also not usually lethal.<sup>2</sup> Brawls are episodes of combat between multiple, often unrelated participants; the term does not imply lethal outcomes. These deaths occurred between unrelated victims without domestic violence and were committed using one sword; they are best described as mutual homicides, including an immediate revenge homicide.

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### Mutual Homicide, Immediate Revenge Homicide, Sharp Force Injuries



## **H91 Mob Justice Fatalities: Case Studies**

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**WITHDRAWN**

## H92 Woman-to-Woman Sexual Violence: A Case Discussion

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**Learning Overview:** After attending this presentation, attendees will be aware of the lesser-known entity of woman-to-woman sexual assault.

**Impact Statement:** This presentation aims to provide an insight and create awareness into the lesser-known and even lesser-understood entity of female-to-female or lesbian sexual assault.

The term “sexual assault” is used to refer to any unwanted contact and behavior that is perceived to be of a sexual nature and which takes place without consent. It has been used here as an umbrella term to represent the different ways women may refer to their experiences. Even in the Lesbian/Gay/Bisexual (LGB) population, surveys conducted focus on male-perpetrated rape of victims who are female. Because of deeply rooted assumptions about gender role and notions about the maternal and caregiving nature of women, it is commonly assumed that women are incapable of inflicting violence on another person. Women tend to assume they are safe or at a significantly reduced risk of being sexually violated when in the company of other women.<sup>1</sup>

The lack of recognition of women as offenders is reflected widely in various statistics worldwide. Due to homophobia and discrimination, the women generally do not come forward. There may also be a strong need for unity and holding onto same-gender relationships leading to concealment of the rape incident.<sup>2</sup> It may also be difficult for women to frame criminal charges against female perpetrators, due to the different legal definitions of rape between countries and criminal jurisdictions. There is also a lack of same sex resources.<sup>3</sup> Further research is needed to understand the motivations and ramifications of sexual violence between women. Service providers should be educated about the possibility of same-sex sexual violence and ways to help survivors to recover from the trauma, connect with others, and confront multiple oppressions.

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### Sexual Assault, Woman-to-Woman Assault, Same-Sex Violence

### H93 Fatalities Due to the February 2021 Winter Storm in Harris County, Texas

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**Learning Overview:** After attending this presentation, attendees will have learned about fatalities in Harris County, TX, during the February 2021 winter storm.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview of the deaths encountered during a major winter storm.

During this timeframe, there is an average daytime high of 39°F and low of 25°F.<sup>1</sup> Due to the lack of cold weatherization of the independent Texas power grid and the inability of Texas to import electrical power from the national power grids, 91% of Harris County residents lost power at some point during the week.<sup>2</sup> The freeze also caused water pipes to malfunction, leaving 65% without running water.<sup>2</sup> Reviewed here are 41 fatalities during the storm that were examined at the Harris County Institute of Forensic Sciences from February 12 to February 23, 2021.

Hypothermia is clinically defined as a core body temperature of less than 95°F. The postmortem diagnosis of hypothermia is a challenge as many of the findings are non-specific. The most commonly reported are gastric petechiae (Wischnewski spots), cherry-red lividity, excoriation of the nose, ears, and hands, and acute hemorrhagic pancreatitis. Scene investigation and circumstances are key to the diagnosis.

Of the 41 fatalities, 23 were autopsied, 13 were externally examined, and 5 were reported but not examined. Causes of death included hypothermia, carbon monoxide poisoning with hypothermia ( $n=5$ ), and one blunt trauma case. All manners of death were accident. Twenty-five decedents were male, 16 were female, and the average age was 62 years old, with age ranging from 7 to 100 years old. Their ethnicities were White ( $n=17$ ), Black ( $n=16$ ), Hispanic ( $n=7$ ), and Asian ( $n=1$ ). Thirty-five deaths were on scene and 6 were hospital deaths (interval to death ranging from 2 minutes to 16 hours after arrival). Deaths of those with fixed residences was much greater ( $n=31$ ) than those that were homeless ( $n=10$ ). Average axillary temperatures of the bodies on scene were 63.6°F and ranged from 41°F to 90.6°F. The most common co-morbidity was hypertension ( $n=15$ ). The most common autopsy findings were Wischniewski spots in the stomach ( $n=12$ , 30%). The presence of ethanol on postmortem blood analysis was the most common toxicology finding ( $n=11$ , average: 0.175g/dL).

Hypothermia is a major preventable cause of mortality during severe storms, and global climate change will likely make these weather events more common. The prolonged loss of electrical power infrastructure can lead to improper use of portable generators or indoor grilling for heating purposes increasing the risk of carbon monoxide exposure. The risk of hypothermia in vulnerable groups, such as the elderly, is also much greater. Identification of these groups and correct classification of these deaths by medical examiners are important for public health data, targeted education, and intervention.

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#### Hypothermia, Forensics, Autopsy

## H94 COVID Testing and Postmortem Interval

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**Learning Overview:** After attending this presentation, attendees will understand the procedure of testing for COVID-19 on postmortem cases. Attendees will gain a better understanding of the viability of the virus.

**Impact Statement:** This presentation will impact the forensic science community by encouraging other medical examiner's offices to test their decedents for COVID-19 to minimize the exposure to their staff. This is of great importance when considering variants and the exposure rate on scenes and in the office.

Between January 2020 to April 2021, there have been approximately 578,520 deaths due to the coronavirus in the US.<sup>1</sup> According to the National Association of Medical Examiners, there is no uniform way of investigating COVID-19 deaths by medical examiners.<sup>2</sup> These deaths are traditionally not under the jurisdiction of the medical examiner. Each office will vary on their response to COVID-19 deaths.

The Washington, DC, Office of the Chief Medical Examiner (OCME) has taken jurisdiction over all COVID-19 deaths that occur in the District and performs testing on decedents suspected of having it. If it is reported to the investigator that the decedent has had COVID-19 symptoms or was recently exposed, it is documented in the case synopsis. An X-ray is then completed. If the medical examiner is suspicious, a nasopharyngeal swab or lung swab (during autopsy) is taken from the decedent and sent to the DC Public Health Laboratory.

This study reviews the postmortem intervals between pronouncement time of death, time of testing, location of death, and more. DC OCME has swabbed 347 decedents for COVID-19 from March 2020 through April 2021. It has been determined that 56 cases came back positive. It was found that about 60% of decedents died at home, 17% at a hospital, 9% outside, 8% at a nursing home, and 3% at a shelter or hotel. Approximately, the decedents were pronounced on an average of 35.8 hours prior to being swabbed for COVID-19. It was also found that decedents were transported and placed in the freezer 29.9 hours before being swabbed. Interestingly, decedents were last seen alive, on average, four days before swabbing, with one decedent in a moderate stage of decomposition.

This research provides statistical data that the coronavirus is potentially still active even when being exposed to a harsh, cold environment (i.e., the freezer) and to decomposition changes. Depending on the circumstances surrounding the death, it is advised to test decedents for COVID-19 to decrease risk of exposure to staff members. This will help to obtain accurate numbers pertaining to COVID-19 deaths.

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## COVID-19, Pandemic, Autopsy

## H95 SARS-CoV-2 Active Replication in Cadavers

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the actual biological risk related to SARS-CoV-2 infection both in Forensic autopsy Cases (FCs) and in Clinical autopsy Cases (CCs).

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of SARS-CoV-2 active replication in cadavers and of the persistence of high viral loads even several days after death. The key message will be that, despite the exposure, full compliance with guidelines and safety standards can guarantee health preservation in the autopsy room.

During the ongoing pandemic, autopsies have represented and represent a valuable tool to assess the cause of death in patients affected by SARS-CoV-2 infection, providing data of great epidemiological and research interest.<sup>1-4</sup> However, the actual biological risk of the pathology staff during autopsies on SARS-CoV-2-confirmed cadavers has not been precisely assessed, albeit some evidence on the persistence of relatively high viral loads after death has been reported.<sup>5,6</sup>

This study performed microbiological testing on the postmortem swabs of 17 FCs and 12 CCs admitted to the Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome, Italy. All CCs died during hospitalization, while only 7/17 FCs died in a hospital. Swab samples were collected from the nasopharyngeal tract and/or lungs of cadavers (registering the interval of time between death and sample collection) and, every two weeks, naso-pharyngeal swabs were also performed on all the dissectors. On the postmortem samples, this study performed a multiplex real-time Polymerase Chain Reaction (PCR) to detect three SARS-CoV-2-specific genes (RdRP, S, and N), a gene of the Sarbecoronaviruses (E), and an in-house real-time PCR assay to detect subgenomic messenger RNAs (mRNAs) (intended as indicators of viral active replication). Hence, this study quantified viral load. It was found that, albeit the mean postmortem interval was higher in FCs than in CCs (5.93 days vs. 2.25 days), the mean viral load of FCs was higher than that of CCs (1.02 1011 copies/mL vs. 6.52 108 copies/mL). Moreover, in 13/29 cases, evidence of active replication was found. These findings confirm the persistence of significant viral loads even several days after death. Moreover, there was a statistically significant (p-value <0.05) relationship between death without hospitalization and the presence of active viral replication. This study did not find a statistically significant relationship between swab site and detection of subgenomic mRNAs.

In the light of scientific literature and these results, full compliance with guidelines to contain biological risk in the autopsy room must be considered critical. In fact, following these guidelines and operating in a Biosafety Level-3 (BSL-3) autopsy facility, in this team there were no cases of infection among the dissectors.

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### SARS-CoV-2, Viral Replication, Viral Load

## H96 The Postmortem Persistence Time of Sars-CoV-2 Virus: An Analysis of a Case Series

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**Learning Overview:** After attending this presentation, attendees will understand the possibility of postmortem persistence of the SARS-CoV-2 virus.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the results of an operative protocol with postmortem swabs performed on a case series.

**Introduction:** Postmortem activity of the SARS-CoV-2 virus has been poorly studied. From a social and cultural point of view, the absence of these data led the institutions, preventively, to prohibit the funerals of infected bodies and initially to reduce the number of COVID-19 autopsies.<sup>1</sup> However, very few studies have evaluated the virus persistence in the body. The presentation proposes an operational protocol with the execution of postmortem swabs to understand the persistence time of the virus based on the time from death.

**Materials and Methods:** The sample included 25 patients who died at the COVID-19-Center-Mater Domini of Catanzaro with incoming diagnosis of positivity to the SARS-CoV-2 test.

The period of hospitalization, medical records, comorbidities, and cause of death were analyzed for each patient. Nose-pharyngeal, oropharyngeal, and endo-bronchial swabs (only in autopsy cases) were performed postmortem at serial timings starting from the moment of death up to 24 hours (0-2-4-6-12-24hrs). The swabs were subjected to one-step Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) with Cycle threshold (Ct) values, using as targets the gene markers for the E, RdRp (RNA-dependent RNA polymerase), N proteins of the virus. In some cases, this method was combined with the use of immunofluorescence technology for the rapid detection of the N antigen (nucleocapsid protein) in order to verify the presence of both viral antigens and RNA.

**Results:** The analysis of the results of the swabs showed a variable trend in positivity in relation to the time elapsed since death. In particular, postmortem permanence of the SARS-CoV-2 virus in the corpse in about 52% of cases was detected. Repetition of the swab over time showed that there was a positivity in the various timings examined, with 12/25 cases still positive 24 hours after death. The persistence of the virus did not show correlations with the comorbidities from which the patients were affected or with specific cause of death. None of the health care workers involved in autopsies tested positive for the swab.

**Discussion:** The proposed protocol demonstrates that death is a biological phenomenon which, despite abiotic processes, may not inactivate the virus in the corpse even beyond 24 hours after death. In particular, the positive result of the swabs demonstrates the possibility of the virus to survive for a long time in the corpse. The data obtained allow us to hypothesize resistance of the pathogen to postmortem changes in temperature and acid-base balance.

By virtue of the results obtained, the choice not to expose the COVID-19 corpses appears to be acceptable and scientifically valid. From a health point of view, the choice not to carry out autopsies is now not scientifically supported, although an expert team with safe autopsy rooms and suitable devices is needed. An increase in autopsies is desirable in order to investigate the pathogenetic mechanisms of the virus on humans for diagnostic, therapeutic, and preventive purposes.

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### Forensic Science, SARS-CoV-2, Postmortem Swab

## H97 Ventilated Postmortem Imaging of COVID-19-Related Lung Disease With Clinicopathologic Correlation

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential applications of radiology to death investigation and the type of information/data that may be extracted through radiologic-pathologic correlation.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that ventilated Postmortem Computed Tomography (PMCT), combined with limited autopsy, can provide insight into the pathophysiology of COVID-19-related lung injury and the differences between COVID-19 and non-COVID-19 injury.

The emergence of the COVID-19 pandemic inadvertently revealed an opportunity to expand the application of forensic imaging methodologies. COVID-19, caused by SARS-CoV-2, is associated with lung injury, Acute Respiratory Distress Syndrome (ARDS) and high mortality.<sup>1,2</sup> Early in the pandemic, the virulence of COVID-19 and its mechanisms of transmissibility were incompletely understood, resulting in variable recommendations for protective measures and anxiety in those in and out of the health care setting. Autopsy—a vital part of cause-of-death investigation—was suspended in many hospitals around the world due to the inherent risk of disease exposure, resulting in a loss of potential data and a delay (if not a void) in understanding of the disease pathophysiology and mortality.<sup>3</sup> Albeit necessary at the time to protect health care workers, the resultant deviations from standard practices highlight the need to explore supplemental techniques for Postmortem (PM) investigation, such as PM imaging for characterization of COVID-19 and non-COVID-19 lung disease, which may help explain why studies evaluating ARDS secondary to COVID-19 and comparing it to non-COVID-19 ARDS have reported mixed findings and conclusions, including the potential for various phenotypes along a spectrum of COVID-19-related lung disease.<sup>4-14</sup> While published reports describe PMCT findings in COVID-19 patients, PMCT may inform on disease pathophysiology, particularly when combined with ventilation studies, pressure flow analysis, and autopsy findings.

Two female patients who required ventilation for respiratory failure were examined: one died of complications of COVID-19, and the other died from cerebral hemorrhage, but not before developing ventilator-associated pneumonia. Both patients underwent PMCT within 48 hours of death (Discovery 570c NM/CT, GE Healthcare), imaged at Positive End-Expiratory Pressures (PEEP) of 0, 10, 20, and 30cm H<sub>2</sub>O. Using 3D Slicer software, the lungs were isolated and segmented by degree of aeration: over-inflated (<-1000 to -901 HU, 95% aeration); well-inflated (HU -900 to -501 HU, 70% aeration); and poorly inflated (-500 to -101 HU, 30% aeration). Targeted autopsies were conducted.

Quantitative analysis of PMCT showed that total aerated lung volumes normalized by height were lower at rest (0 cm H<sub>2</sub>O) in the COVID-19 patient compared to the non-COVID-19 patient. Concordantly, normalized lung weights were greater in the COVID-19 patient (807.2g/m vs. 629.9g/m). Pathology demonstrated congestion, acute fibrinous organizing pneumonia, and scattered accumulations of neutrophils within smaller airways and alveoli in the COVID-19 patient, and occasional foci of intra-alveolar edema and hemorrhage in the non-COVID-19 patient. PMCT-derived pressure-volume curves showed relatively increased lung compliance in the COVID-19 patient.

This study reports a novel comparison of ventilated PMCT findings in a COVID-19 and non-COVID-19 patient with autopsy findings. Although the COVID-19 patient demonstrated edematous lungs with lower aerated volumes at rest, lung compliance was preserved if not increased, a surprising finding consistent with some *in vivo* studies showing intriguingly high lung compliance in COVID-19-related ARDS.

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### COVID-19, Forensic Imaging, Radiologic-Pathologic Correlation



## H98 Postmortem Computed Tomography (PMCT): A Valuable Tool in the Study of SARS-CoV-2-Related Deaths?

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the role of the PMCT in cases of confirmed/suspected SARS-CoV-2-related deaths.

**Impact Statement:** This presentation will impact the forensic science community by presenting the evidence of a correlation between radiological and histopathological findings in cadavers with microbiological evidence of SARS-CoV-2 infection and a previous clinical diagnosis of COVID-19.

Since the first wave of the SARS-CoV-2 pandemic, Computed Tomography (CT) has been proposed as primary diagnostic tool for COVID-19 cases. The most common pulmonary CT findings in these patients are bilateral lung involvement with ground-glass opacity, thickened interlobular septa, vascular enlargement, air bronchogram, bilateral lower lobe involvement, and complete consolidation of both lungs.<sup>1</sup> PMCT imaging has been proposed as a screening method for SARS-CoV-2 infection before autopsy and has been employed in forensic investigations of confirmed/suspected SARS-CoV-2-related deaths.<sup>2-6</sup>

The aim of this study is to compare the thoracic PMCT imaging and the histopathological findings in eight cadavers with microbiological evidence of SARS-CoV-2 infection and clinical diagnosis of COVID-19. Cadavers underwent a PMCT, then a full forensic autopsy with histopathological examination. At thoracic PMCT, in 50% of cases and in 25% of cases, respectively, a massive consolidation and crazy-paving pattern were found that corresponded to severe lung COVID-19 disease at the histopathological analysis. In 25% of cases, lung PMCT showed limited upper ground-glass opacities due to hypostasis. The cause of the death in 75% of cases was a cardiorespiratory failure with serious pulmonary histopathological signs of COVID-19, while in the 25% of cases histopathological analysis revealed non-specific COVID-19 lung alterations (then, the cause of death was not attributed to SARS-CoV-2 infection).

In forensic science, thoracic PMCT can improve diagnostic sensitivity in cases of SARS-CoV-2 lung impairment.<sup>7</sup> Lung PMCT sensitivity is enhanced by the natural contrast caused by air; however, a possible bias of this radiological technique is represented by hypostases, which can appear as areas of greater opacity, frequently of the ground-glass type, distributed in lower regions of the lungs.<sup>8</sup> That being said, at PMCT, cadavers with a diagnosis of COVID-19 usually have suggestive lung alterations (massive and bilateral consolidations, “crazy-paving pattern,” upper areas of ground glass opacity in the context of hypostatic ground glass opacity in both lungs) that can be differentiated by hypostases. However, despite the improvements given by this emergent radiological approach, the gold standard for postmortem studies of SARS-CoV-2-positive cadavers is still represented by autopsy and histopathology.<sup>2-4,6</sup> Therefore, in SARS-CoV-2-positive cadavers, evidence of a good correspondence between the lung involvement revealed by PMCT imaging and that found at histopathological analysis (as reported in this study) can be important for forensic practice.

Finally, these findings highlight the importance of performing thoracic PMCT when data of SARS-CoV-2 infection are available, suggesting that, in future, this technique could become a useful and reliable tool to attribute the cause of death to severe lung COVID-19 disease.

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### Postmortem CT, COVID-19, Forensic Pathology

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## H99 COVID-19-Associated Myocarditis at Autopsy

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**Learning Overview:** After attending this presentation, attendees will better understand the histologic spectrum, significance, and frequency of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) -associated myocarditis at autopsy.

**Impact Statement:** This presentation will impact the forensic science community by aiding autopsy pathologists in diagnosing, characterizing, and interpreting the likely significance of myocardial inflammation in COVID-19 deaths.

While the respiratory complications of COVID-19 have been well-publicized, an increasing body of evidence suggests associated cardiac injury may also play a role in morbidity and mortality. There are multiple ways by which SARS-CoV-2-associated myocardial injury may occur, including myocarditis. Reports regarding the incidence of COVID-19-associated myocarditis range widely, however, from 3–55%.<sup>1,2</sup> Additionally, the composition of the myocardial inflammatory infiltrate appears variable, and the potential contribution of myocarditis to death is unclear.<sup>1</sup>

A retrospective search was performed of the electronic autopsy database at the University of Wisconsin Hospitals and Clinics, utilizing search terms “COVID” and “SARS,” yielding 209 cases. Report review revealed 23/209 (11%) to have had positive nasopharyngeal SARS-CoV-2 Polymerase Chain Reaction (PCR) testing antemortem, postmortem, or both. COVID-19 was determined to be the cause of death in 10/23 (43%) cases and incidental in the remaining 13. Two of the incidental cases were external examinations only. Of the 21 cases in which internal examination was performed (including procurement of cardiac histologic sections), myocarditis with associated myocyte necrosis was present in 3/21 (14%); in 2/21 (10%), a rare small focus of interstitial lymphocytic inflammation without associated myocyte necrosis was found. All myocarditis cases arose in patients who ultimately died of COVID-19. Immunohistochemical staining for CD68 (macrophage marker), CD3 (T lymphocyte marker), and CD20 (B lymphocyte marker) was performed to better characterize the cellular infiltrate in the myocarditis cases and highlighted a relative macrophage predominance with lesser numbers of lymphocytes. A few scattered neutrophils could also be seen. It has been proposed that migration of infected pulmonary macrophages and/or transient viremia are the mechanisms by which SARS-CoV-2 myocarditis may arise.<sup>3</sup>

In terms of distribution, myocarditis was limited to the right ventricle in two cases. As the lungs are the major organ targeted by SARS-CoV-2, it is suspected that the right ventricle may be at greater risk of impairment/failure than the left, in cases of myocarditis. The remaining myocarditis case was fulminant involving both ventricles as well as the atrioventricular node. *In Situ* Hybridization (ISH) for SARS-CoV-2 spike and nucleocapsid RNA was positive within cardiomyocytes in the fulminant myocarditis case; testing is on-going in the remaining two cases. In 2/3 cases of SARS-CoV-2-associated myocarditis diagnosed at autopsy, both patients appeared to have been improving with stabilized ventilatory requirements prior to precipitous cardiorespiratory collapse.

Encouraging autopsy in such scenarios may thus assist in elucidating the cardiac sequelae of COVID-19 infection. Ultimately, definitive diagnosis of myocarditis requires histologic examination of tissue in order to exclude differential cardiac diagnoses (e.g., stress-induced cardiomyopathy).

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### COVID-19, SARS-CoV-2, Myocarditis

## H100 Anosmia and COVID-19: A Search for the Virus in the Brain Through Immunohistochemical Investigations With the Anti-SARS-CoV-2 Antibody

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**Learning Overview:** This presentation aims to support the search for the cause of anosmia in patients affected by the SARS-CoV-2 virus. Through immunohistochemical investigations, we have highlighted the presence of the virus in postmortem brain samples from areas involved in the olfactory system.

**Impact Statement:** This presentation will impact the forensic scientific community by showing the presence of the SARS-CoV-2 virus in the meninges and around the nerve cells of the olfactory system. Immunohistochemistry also plays a central role in studying the mechanism of infection in the future.

The SARS-CoV-2 pandemic has upset everyone's lives. Each person had to change their lifestyle habits to avoid viral infection. In these two years, thanks to the rapid progress of research, scientists have understood the viral pathogenetic mechanisms and have developed new vaccines. SARS-CoV-2 presents numerous symptoms, often completely non-specific, such as cough, fever, dyspnoea. Anosmia and ageusia are peculiar symptoms of this infection. Patients suffering from COVID-19 suddenly lose the sense of smell in 38.2% of cases and of taste in 36.6%.<sup>1</sup> These senses are recovered spontaneously after two or three weeks, in some cases after several months.

The pathogenetic mechanism underlying the loss of smell is not known. The virus could attack the nerve cells of the nervous and olfactory system, as happens in other organs through the ACE2-receptor. The olfactory dysfunctions induced by the coronavirus can be a direct effect of the virus on the function of the supporting cells, or it can cause metabolic dysfunctions of the olfactory system receptors, as the supporting cells have trophic functions.<sup>1</sup>

In an experiment with hamsters, the experimenters observed desquamation of the olfactory epithelium, infection of supporting cells (preferred to neurons), and intense immune response.<sup>2</sup> Indeed, olfactory epithelium damage can be aggravated by an inflammatory response, leading to cell death. Loss of smell may be due to olfactory bulb inflammation triggered by virus infection. SARS-CoV-2 can infect the central nervous system through the synapses, using the olfactory nerve afferents to reach the olfactory bulb, raising the possibility of SARS-CoV-2 utilizing this infection path as well.<sup>3</sup> Fourteen cases of deceased infected by the SARS-CoV-2 virus were selected from the autopsy case histories of the Institute of Forensic Medicine of University of Foggia. The causes of death were not necessarily infectious.

In each autopsy, whole brain was taken, then fixed in formalin; samples of each organ were also taken. Once fixed in formalin, in the brain have been identified and sampled the olfactory striae, the amygdala, the olfactory trine, and the thalamus. These areas are a part of the olfactory system: from the olfactory bulb originate the striae, which form the olfactory trine. The striae carry the olfactory nerve fibers toward the primary olfactory cortex and the amygdala. The amygdala and the thalamus are part of the limbic system; these areas play an important role in evoking memories and emotions related to smells. All samples are stained with Hematoxylin-Eosin (H&E) and anti-COVID-19 antibodies and examined with an optical microscope. This study found widespread positivity of anti-COVID-19 in brain samples, with perineuronal accumulations of the autoantibody. In the collection of olfactory striae, positivity is present at the level of the meninx.

These findings could support the hypothesis that the virus infect the olfactory nerve cells by entering through the olfactory nerve. These first encouraging results may guide research by evaluating whether the virus that has reached the brain induces an inflammatory response only hypothesized so far. The scientific community should investigate the presence of inflammation through further immunohistochemical investigations.

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### SARS-CoV-2, Olfactory System, Anosmia

## **H101 Died From or Died With COVID-19? The Application of Frailty Scores in the Assessment of Causality in 41 Autopsy Cases in Elderly Community Settings**

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**Learning Overview:** Attendees to this presentation will consider the opportunity to integrate autopsy findings with a clinical score of frailty in the assessment of causality in the elderly population with SARS-CoV-2 disease living in community settings and affected by multiple severe comorbidities.

**Impact Statement:** The aim of this presentation is to validate the methodological approach used to establish the causal relationship between SARS-CoV-2 infection and death in the elderly population.

In the first half of 2020, a new form of coronavirus spread around the world starting from China. The elderly were the population most affected by the virus around the world, in particular in Italy, where more than 90% of the deceased were people over 65 years of age.<sup>1</sup> In elderly populations living in community settings who died in the few weeks in the first wave of the COVID-19 pandemic in Italy, establishing the causal role of COVID-19 infection was extremely difficult due to the multiple and severe comorbidities and the early “lockdown” of autopsies.<sup>2-6</sup> In this scenario, the criteria proposed from the health authorities were inconsistent in the forensic field.

This study proposed the results of 41 autopsies performed from the beginning of May to the beginning of June 2020 in the elderly population living in residential care homes who died in the first few weeks of the first wave of the COVID-19 pandemic with positive swab for virus SARS-CoV-2. Demographic and clinical information were extracted for each subject from their personal records. Postmortem examination was performed many weeks after death (38 days average) and included a multiple swabs panel (oropharyngeal, nasopharyngeal, tracheal, bronchial, and rectal), macroscopic and microscopic examination of tissue samples by routine, and immunohistochemical stainings. Samples for electron microscopy were also collected with surprising results.

The subjects included in this study were 14 men and 27 women; each one was a guest of a nursing home in the city of Trieste. Mean age was 90 years and mean postmortem interval was 38 days, with bodies preserved in different conditions, from cold rooms to refrigerated rooms. Comorbidities were represented by walking impairment/bed rest syndrome (86%), followed by severe cognitive impairment (56%), ischemic heart disease (50%), Chronic obstructive pulmonary disease (COPD) (43%), chronic kidney failure (33%), arrhythmogenic heart disease (26%), hypertension (23%), neoplastic disease with dissemination (23%), diabetes mellitus (13%), and congestive heart failure/valvulopathy (10%). Swabs were analyzed by real time Polymerase Chain Reaction (PCR) and resulted positive in 34 cases (83%). In 53% of cases, acute respiratory failure was indicated as the cause of death, and diffuse bronchopneumonia was observed in 46% of these, 23% in association with diffuse alveolar damage. In 37% of cases, acute cardiovascular failure in ischemic heart disease was indicated as the cause of death, and diffuse bronchopneumonia was observed in 20% of these. Ten percent of deaths were related to cancer, sepsis, and digestive hemorrhage. The Geriatric Index of Comorbidity (GIC) based on the severity of the diseases was used to measure the frailty and vulnerability associated with aging decline to “weigh” the degree of (forensic) causality of death with COVID-19 infection.<sup>7</sup> The probability of a causal relationship was high in 16% of cases, moderate in 14% of cases, low in 47% of the cases, and a certain alternative cause of death, regardless of the outcome of the swab, was found in 23% of the cases.

This presentation introduces a new methodological approach in the forensic assessment of the causality integrating collected autopsy findings with the GIC and alerts pathologists to the importance of a complete postmortem examination in certain or suspected COVID-19-related deaths.<sup>8-10</sup> A brief discussion is also reserved regarding the positivity of swabs collected with a mean postmortem interval of 38 days.

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### COVID-19, Cause of Death, Elderly

## H102 Pediatric SARS-CoV-2 Infection in the Forensic Setting

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**Learning Overview:** Attendees will recognize and cite the elements of the spectrum of SARS-CoV-2 infection among children in the forensic setting.

**Impact Statement:** Forensic pathologists may encounter and thus must be aware of SARS-CoV-2-infected infants presenting as “Sudden Unexpected Infant Death (SUID),” as well as cardiovascular collapse in SARS-CoV-2-infected school-age children with underlying medical conditions.

Complete forensic investigation of unexpected deaths in infancy and childhood includes microbiologic testing for underlying infectious causes. Since March, 2020 (the era of COVID-19), nasopharyngeal swabbing for SARS-CoV-2 was added to supplement the routine screening for other viruses such as enterovirus, respiratory syncytial virus, rhinovirus, and others known to cause pediatric deaths. Over a 16-month period (through June 2021), eight non-traumatic pediatric cases (newborn through age 18 years) were referred to this study’s office, with antemortem (in emergency department) and/or postmortem results positive for SARS-CoV-2. The cases distributed into two groups: (1) infant deaths ( $n=4$ , ages 7 weeks to 4 months); and (2) school-age children ( $n=4$ , ages 5-15 years).

All infants were found unresponsive after a sleep period and in unsafe sleeping environments (i.e., as SUID); attempted resuscitations were unsuccessful. Of the infants, one had sickle cell disease, and he and his parents had influenza-like symptoms in the week prior to death; at autopsy, he had platelet and fibrin microthrombi in the lungs and interstitial pneumonitis. Another had been treated at birth for presumed congenital syphilis but had no recent illness (certified as undetermined cause and manner of death). One infant had “recent rhinitis” but died with evidence of positional asphyxia (manner of death, accident). Neuropathologic examination was notable in one infant for perivascular vacuolization and gliosis, as well as sparse perivascular lymphocytes, similar to changes in adults with COVID-19.<sup>1</sup>

All four school-age children had at least one underlying medical condition (e.g., obesity, metabolic disease, and/or autism) and presented with influenza-like illnesses; three had laboratory findings of multisystem inflammatory syndrome upon testing in the emergency setting. Two children collapsed and died before admission, and the other two were hospitalized, receiving mechanical ventilation and Extracorporeal Membrane Oxygenation (ECMO). One of the hospitalized children developed a complication of ischemic stroke ipsilateral to the ECMO cannula (total hospital course, 11 days), and the other required abdominal surgery for an ingested foreign body (total hospital course, 4 days); the latter child also had co-infection with adenovirus.

Autopsy findings in the school-age children included acute lung injury/pneumonia ( $n=3$ ), with microthrombi in one. One child had a body-mass index of 45.8, with diabetic ketoacidosis, cardiac hypertrophy, and fatty liver disease; he also had co-infection with adenovirus. One hospitalized child had coronary and cerebral arteritis and aortitis, resembling Kawasaki disease. The other hospitalized child had known neuronal storage disorder, also with an enlarged fatty liver. Nervous system findings were particularly interesting: all had subtle evidence of meningoencephalitis, focally involving the brainstem, as reported in adults with COVID-19.<sup>1</sup> Of note, two children had heretofore unreported histologic evidence of autonomic/peripheral nervous system involvement by mononuclear inflammation, raising an additional possible neurologic contribution to cardiovascular (autonomic) collapse.

In conclusion, SARS-CoV-2 may present very differently in infants (more “SUID-like”) than in older children, who, as in the adult population, often have underlying medical conditions predisposing to fatal outcomes, and may also have co-infection with a second virus. Kawasaki-like findings may occur in a subset. Neuropathologic changes in children resemble those in adults. Additional analysis of the peripheral/autonomic nervous system of children with COVID-19 would be of interest in understanding the propensity for cardiovascular collapse/sudden death in the population coming to forensic autopsy.

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### COVID-19, Sudden Unexpected Death in Infancy, Sudden Unexpected Death in Childhood

## H103 Overdose Analysis in a Year of COVID-19 in Orange County, California

*Kelly Keyes, BS\*, RTI International, Research Triangle Park, NC; Chelsea Brown, MA, Orange County Sheriff Coroner, Santa Ana, CA*

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**Learning Overview:** After attending this presentation, attendees will understand how the COVID-19 (also called SARS-CoV-19 or Coronavirus disease 2019) pandemic affected overdose rates at a large coroner's office, specifically Orange County, CA.

**Impact Statement:** This presentation will impact the forensic science community, especially those involved in medicolegal death investigations, by presenting information on how a pandemic affected rates of death in cases not directly related to the pandemic, focusing on overdose deaths, from March 2020 when lockdown from the pandemic began, until a year later in March 2021.

Early in the pandemic, there was much publicity about anticipated effects of the pandemic and associated lockdowns on the population and mental health. As the pandemic progressed there was much information released about the skyrocketing rates of overdose deaths during this time frame. Unfortunately, we still remain too deep in the crisis to fully understand any long-term effects on our populations, including on mental health, financial stressors, and other changes in people's lives, but we do have information available on those dying in this initial year of the pandemic.

This presentation aims to examine the potentially indirect consequences of the pandemic on death rates at a medicolegal death investigation office; consequences that in addition to the burden of increased deaths directly attributed to the virus, adds significantly to the workloads of the staff at these medical examiner and coroner offices. The added workload is felt at all levels of the office, from records to investigation to autopsy. Along with other indirect pandemic deaths (such as pandemic-related suicides and natural deaths complicated by lack of treatment during the pandemic), this presentation will focus on increased overdose deaths within the jurisdiction. This presentation will also briefly address all classifications of death, including traffic-related fatalities and deaths by suicide, during this time frame as well.

This presentation will include comparisons to rates from the prior five years to examine the relationship of potential trends. Analysis will be done on various age groups, races, and genders. This presentation will also look at the specific substances that the cause of death is attributed to, types of substances (licit vs. illicit, when it is known), manners of death for these overdose cases, as well as locations of injury.

This presentation can serve as a guideline for potential future resource allocation in times of pandemic, as well as serve a public health role for future evaluation to save lives should we as a nation again be faced with such challenges. This presentation can also be used by medicolegal death investigators to have heightened awareness of death rates during a pandemic.

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### Medicolegal Death Investigation, COVID-19, Overdose

### H104 A Year of Mandatory, Universal COVID-19 Postmortem Testing in San Francisco: Lessons Learned

*Rand Miyashiro, MA\*, City and County of San Francisco, San Francisco, CA; Willi McFarland, MD, PhD, Department of Public Health, City and County of San Francisco, San Francisco, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the utility, processes, and likely outcomes of universal postmortem testing mandates in response to pandemic situations such as COVID-19.

**Impact Statement:** This presentation will impact the forensic science community by providing a case study for the how, when, where, who, and why of universal postmortem testing in the event that such public health mandates are implemented for future surges of COVID-19 or during similar emerging pandemics. Findings may help pathologists develop guidance for COVID-19 postmortem test orders where testing capacity is limited. This includes a greater understanding of COVID-19-positive persistence of greater than three days postmortem.

The City and County of San Francisco is the only United States health jurisdiction to have required universal postmortem COVID-19 testing, per research. The rationale for the mandate was to: (1) help protect first responders and persons working in funeral-related services; (2) identify missed cases of COVID-19, including those without symptoms, for contact tracing; (3) understand who is at highest risk for death due to COVID-19; and (4) provide the public with information on the COVID-19 epidemic. The mandate was in effect from June 2020 through June 2021, a period covering peak mortality and transmission. By emergency order of the county Health Officer, all decedents were required to be tested and positive results reported to the Department of Public Health. To assist the community in meeting this requirement, San Francisco implemented a specialized Decedent Testing Unit (DTU), in collaboration with the medical examiner, to rapidly respond to the COVID-19 testing needs of all locations where deaths occurred, including facilities with limited postmortem testing capacity. Based on under 3,000 tests and just under 90 positive results, this order has shown that COVID-19 was detected in many cases when tested 72 hours or more after death. During a year of testing, positivity rates ranged from 0–14% using a 14-day rolling average. Positive cases included asymptomatic as well as symptomatic decedents who had not sought medical attention. The detection of otherwise undiagnosed cases, primarily in at-home and hospice deaths, confirmed the concerns of first responders, such as emergency medical staff, who were proponents of the program. This presentation will discuss testing among place-of-death locations (e.g., hospitals, homes) and compare the percentage of positive decedents against the relative frequency of place of deaths within San Francisco and California.

Complementing well-documented COVID-19 mortality rates occurring in hospitals and skilled nursing facilities, this presentation will present positive rates for homes, outdoors, and other locations of death. The utility of test orders in situations where a decedent was or could be double tested (hospitals and the medical examiner) will also be discussed.

**Conclusion:** Review of results from a year-long county-wide health order mandating universal COVID-19 postmortem testing showed that decedent-positive cases came from more places of death and were detected postmortem for much longer periods than anticipated.

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#### COVID-19 Testing, Postmortem Testing, COVID-19 Postmortem Persistence



## H105 The Challenges in the Disposal of COVID-19- Deceased Bodies in Mumbai, India

*Manoj Parchake MD\*, Seth GS Medical College & KEM Hospital, Mumbai, Maharashtra, India*

**Learning Overview:** Forensic experts will learn the challenges faced while disposing of the COVID-19 deceased bodies in a highly populated city like Mumbai.

**Impact Statement:** This presentation will impact the forensic community by demonstrating how the challenges were overcome while disposing of the COVID-19 deceased bodies in a highly populated city like Mumbai.

On December 31, 2019, the China Health Authority alerted the World Health Organization (WHO) to several cases of pneumonia of unknown etiology in Wuhan City in Hubei Province in central China. The cases had been reported since December 8, 2019, and many patients worked at or lived around the local Huanan Seafood Wholesale Market, although other early cases had no exposure to this market. On January 7, a novel coronavirus, originally abbreviated as 2019-nCoV by WHO, was identified from the throat swab sample of a patient. This pathogen was later renamed as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group and the disease was named Coronavirus Disease 2019 (COVID-19) by the WHO.

The COVID-19 pandemic impacted almost every country on Earth. The current status of COVID-19 cases as per WHO are 216,303,376 and deaths are 4,498,451. In India, the current status of COVID-19 cases and deaths as per WHO is 32,737,939 confirmed cases of COVID-19 with 438,210 deaths. The current status of Covid-19 in Mumbai is total confirmed cases 7, 43,832 and 15,976 deaths, among which 13,617 deaths occurred in individuals above 50 years of age.

Challenges while disposing of the COVID-19 deceased bodies are securing isolation wards for COVID-19 deceased bodies. Problems were faced while shifting COVID-19 deceased bodies from wards to morgues, providing separate mortuary cabinet to preserve the deceased bodies in morgues, and hospitals having to follow government guidelines such as necessary documentation for the disposal of dead bodies. Initially, there was hesitancy of health workers to provide service to dead body disposal. There was a lack of sufficient mortuary cabinets to preserve the Covid-19 dead bodies initially. Challenges were faced while handing over the bodies of COVID-19 decedents to police and relatives. Difficulties were faced on the death of COVID-19 patients or quarantine patients at home. There were difficulties in tracing the relatives of the COVID-19 deceased. Also there was the impact of lockdown, which affected the disposal of Covid-19 deceased. There were long waiting periods for the disposal of COVID-19 deceased bodies for electric cremation at the cemetery in the city. There were challenges in handling the relatives at funeral sites and environmental cleaning.

All these challenges were tackled efficiently by government authorities. For the future, there is a moving morgue concept providing trailer trucks with air-conditioning that can be procured at various morgues to handle the COVID-19 deceased bodies. These trailer trucks can be moved to another hospital as per requirement, which can be very cost effective to the government.

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### Challenges, COVID-19-Deceased Bodies, Mumbai

## H106 Challenges in Managing Dead Bodies With COVID-19 in Indonesia

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**Learning Overview:** After attending this presentation, attendees will better understand the management of infectious dead bodies in Indonesia.

**Impact Statement:** This presentation will impact the forensic science community by giving an update on challenges in managing dead bodies with COVID-19 in Indonesia.

As of August 30, 2021, the COVID-19 pandemic has infected almost 217 million people and killed around 4.5 million people worldwide since its first discovery in December 2019 in Wuhan, China.<sup>1</sup> The COVID-19 pandemic in Indonesia has already infected around 4 million people and resulted in 132 thousand deaths.<sup>2</sup> This increasing death toll poses another threat aside from the fact that health care facilities and the system is on the verge of exhaustion and burnout.

In Indonesia, people who have died with COVID-19 will undergo a specific protocol, as the Indonesian Ministry of Health mandates, before the deceased can be buried in the cemetery. In general, the deceased will be cleaned and disinfected, then wrapped in plastic before being put inside a wooden coffin and being sealed tightly by using another plastic on the outside of the coffin. This procedure is done in a special room for dead body preparation and is done by trained funeral staff using personal protective equipment level 3. During the protocol process, the family cannot be with the deceased because of the risk of infection.<sup>3</sup>

Despite several efforts that the government has made during the pandemic, there is always a slight discrepancy with the peoples' expectations. This is indeed bound to happen because of the diverse background and multicultural nature of Indonesian people. The biggest challenge from managing dead bodies with COVID-19 is the uncooperative families who refuse to let their loved ones undergo the protocol. Eastern culture is still strongly attached in Indonesian people that the deceased must be held in a religious ceremony for the afterlife. So if they abide by the protocol, then the deceased cannot be given the religious ceremony before burial. Another challenge also rises from the limited burial ground, so the government was forced to make a public burial ground for all dead bodies with COVID-19, and there are also challenges in filling out the death certificate correctly. All of these challenges have led to improvements in the dead body management system in Indonesia, despite many shortcomings.

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### COVID-19 Pandemic, Dead Bodies Management, Indonesia

## H107 Estimating the Postmortem Interval (PMI) Using Decomposition Scoring Systems: A Comparative Study

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**Learning Overview:** After attending this presentation, attendees will better understand the predictive reliability of quantitative methods of scoring decomposition used for estimating post-fatal interval

**Impact Statement:** This presentation will impact the forensic science community by it showing limitations and advantages of decomposition scoring procedures for estimating PMI.

Estimating time since death, commonly referred to as PMI, is a crucial task in every death investigation. Unattended deaths or decomposed bodies are usually the most challenging cases for pathologists and anthropologists commonly requested to estimate the PMI. An accurate estimate of PMI may corroborate witness statements, support the credibility of other physical evidence, drive decisions on the allocation of investigative resources, and serve as evidence for subsequent prosecutorial action. Several quantitative methods of scoring decomposition have been suggested for bodies found in aquatic and terrestrial environments. Among them, two approaches are worth of mentioning: Total Body Score (TBS)/Accumulated Degree Days (ADDs) and Total Decomposition Score (TDS)/ADDs. Megyesi et al. introduced first a point-based system for the main three anatomical regions (i.e., head and neck, trunk, limbs) to produce a TBS that is a reliable quantitative value representing the relationship between decomposition and ADDs.<sup>1</sup> This approach has been recently improved by Gelderman et al. who developed an up-dated decomposition scoring method called TDS based on six decomposition stadia for each body region and two different formulas for indoor and outdoor cases.<sup>2</sup>

In the present study, TBS and TDS have been applied on forensic cases with established known PMI. This obtained PMI was then compared with the PMI estimated by using the entomological method for bodies colonized by insects. The sample study was represented by 11 bodies in total (5 females and 6 males) with an age range from 29 to 86 years, mostly discovered indoors (6 out of 11 cases). The range of presumptive PMI was from a minimum of ten days up to about two years. The bodies showed different stages of decay: four bodies were in active decay stage, five were pre-skeletonized, and two were partially mummified. The series of cases were divided into three groups according to the PMI: shorter than 50 days (four cases), between 50 and 100 days (five cases), longer than 100 days (two cases).

For PMI shorter than 50 days, entomology provided an accurate estimation in three out four cases and underestimated in one case. TBS provided an underestimation of the PMI in three cases, and an overestimation in one case up to 23days (1775,23ADD). TDS underestimated the PMI in two cases up to 12days (129,6 ADD) and overestimated the PMI in another two up to 9 days (129,6 ADD). For the five cases with PMI between 50 and 100 days, the distances between the PMI estimates and the real PMI were shorter than the distances found in other cases, although all three methods provided an underestimation of the PMI. For the two cases with PMI longer than 100 days, none of the three methods was reliable. In conclusion, according to these results, the entomological method provided a fairly accurate estimate of the minimum PMI for cases with PMI below 50 days. TDS resulted in being much more reliable than TBS and entomology in estimating PMI between 50 and 100 days.

These results suggest that using the entomological method together with TDS can increase the predictive accuracy in the estimation of PMI, especially in PMI <100 days.

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**Postmortem Interval (PMI), TDS (Total Decomposition Score), TBS (Total Body Score)**

## H108 A Long-Term Study of Decomposition at a Tropical Taphonomy Facility: 2013–2021

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**Learning Overview:** Attendees can expect to be presented the first comprehensive overview of pig decomposition at a tropical decomposition site, including the extent, frequency, and timing of common postmortem processes.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the analysis of a long-term taphonomy database can improve our understanding of decomposition at a given site while stimulating research and applications suitable for use with human remains.

Taphonomy is influenced by several variables that can cause significant variation in the extent, frequency, and timing of postmortem processes. An improved understanding of this variation is the primary goal of decomposition studies. Variation associated with taphonomy in tropical climates is very poorly understood because relatively few decomposition studies have been conducted in the tropics. To address this gap in knowledge, a series of nine carcass decomposition studies were conducted at Chaminade University of Honolulu from 2013–2021. Pig (*Sus scrofa domesticus*) carcasses, three per study, were decomposed on the soil surface or a weighing frame placed on the soil surface. Carcasses weighed approximately 35kg at death and were killed using a range of methods, including electrocution, bolt gun, and exsanguination. Carcasses were placed at the decomposition site within two hours of death and decomposition was monitored for 14 days. Measurements included total body score, mass loss, skin pH, skin temperature, skin oxidation reduction potential (Eh), larval mass pH, larval mass temperature, larval mass Eh, ambient temperature, and ambient relative humidity. All data were compiled and analyzed to identify processes and trends associated with the decomposition of carcasses at the site. The Köppen-Geiger climate classification of the site is Tropical Savanna.

The site is 90m above sea level with mean annual precipitation of 700mm, most of which occurs in the autumn and winter (October–March). The site is rocky with silty clay soil dominated by guinea grass (*Megathyrsus maximus*). Only the small Asian mongoose (*Herpestes javanicus*) has been observed scavenging.

A number of postmortem processes and trends were observed consistently regardless of season:

- All carcasses were a warm temperature (~40°C), slightly acidic/neutral (5.0–7.5pH), with little to no available oxygen (-400–+200 milliVolts: Eh)
- All carcasses were patently bloated within 24 hours postmortem
- All carcasses supported patent fly larval masses within 3 days postmortem
- All fly larval masses migrated from carcasses within 10 days postmortem
- All carcasses lost 75% of their mass within 10 days postmortem
- Other postmortem processes were observed less frequently: 80%–abdominal rupture
- 60%–limb disarticulation 53%–rib exposure
- 40%–mandible disarticulation (17% on weighing frame, 56% on soil)
- 13%–complete loss of soft tissue

The current data show that the long-term analysis of taphonomy data can improve our understanding of decomposition by facilitating the identification of common processes and trends. For example, the decomposition of pig carcasses at the current site can be described as rapid, regardless of season. Bloating was patent within 24 hours postmortem. Color changes and fly larvae were well established within three days postmortem. The temperature, pH, and Eh of carcasses indicated an ideal habitat for microbial and larval activity. Carcasses lost ~75% of their mass and entered advanced decay within ten days postmortem. Carcasses tended to rupture between days 3–9 postmortem but the exposure and disarticulation of the skeleton was less predictable. A complete loss of soft tissue was atypical. Although these data are not necessarily representative of human taphonomy, they have stimulated a number of research projects in partnership with human decomposition facilities and medicolegal death investigation agencies.

### Taphonomy, Carcass, Tropics

## H109 A Longitudinal Study of Human Decomposition Across Summer and Winter in Quebec, Canada

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**Learning Overview:** Attendees can expect to be presented the first longitudinal study of human corpses in a cold, continental climate in Quebec, and to learn which taphonomic patterns to expect in this environment across various seasons.

**Impact Statement:** This presentation will impact the forensic science community by contributing to fill a gap in the current knowledge base on the biological impact of a cold, continental climate on the patterns and rates of decomposition of human corpses and by providing the first frame of reference for forensic taphonomy in Quebec.

**Background and Aims:** The reliability of current methods to measure the decomposition of human remains and estimate the Postmortem Interval (PMI) such as Total Body Score (TBS) and mass loss is influenced by a range of variables. This can include those linked with the environmental and climatic conditions that may undermine the accuracy of the postmortem conclusions presented to the investigators and to court.<sup>1-3</sup> It is therefore essential to ensure that the approaches used in forensic taphonomy are underpinned by robust evidence-based data.

However, there is a gap in the current knowledge base on how cold, continental climates can impact the decomposition process and the patterns of mass loss of human remains and how these may affect our ability to estimate PMI accurately. This study aimed to address this gap in the knowledge through an experimental approach and in so doing, producing a baseline for forensic taphonomy in Quebec.

**Materials and Methods:** An experiment was carried out at the site for Research in Experimental and Social Thanatology (REST), the first human decomposition facility in Canada.<sup>4</sup> REST is located within a mixed woodland area near Trois-Rivières (Mauricie) in Quebec, which has a humid continental climate according to the Köppen-Geiger climate classification. This type of climate implies significant temperature variations across seasons, with cold, snowy winters, and hot, humid summers.

The bodies of ten adult donors donated through the Université du Québec à Trois-Rivières (UQTR) Body Donation Program were analyzed in this study. The selection of donors consisted of five males and five females, ranging from 55 to 93 years old (median age=72). The donors were all deposited undressed, in a supine position, on the soil surface. All corpses were protected by cages to limit animal scavenging.

A combination of qualitative and quantitative approaches derived from the published literature was used to analyze the patterns and rates of decomposition in real time. The following data were collected from and around the donors:

Endogenous data: visual evaluations of the degradation of soft and hard tissues, internal temperature, entomological activity.

Exogenous data: ambient temperature, ambient humidity, soil surface temperature, freeze-thaw-dry of soil (soil moisture measured through calculating dry matter).<sup>5,6</sup>

**Results and Impact:** The results indicate that the distinct combination of heat and humidity over the summer in Quebec led to desiccation of the remains, associated with differential decomposition, which created a protective “shell” that delayed external decomposition. The results from the winter indicate that decomposition progresses during winter, resulting in desiccation of the remains before the typical progression of decomposition stages, such as bloat and active decay. These findings highlight that the taphonomy approaches such as TBS that are currently available may not be suitable for Quebec’s climate, which may introduce uncertainty in PMI estimations.

The results of this study will contribute to strengthening the conclusions that pathologists and forensic anthropologists present to investigators or to the court in Quebec, including in cases that involve missing people from indigenous communities, which have been a pressing issue across Canada. Ultimately, this study will facilitate establishing comparisons with other geographical areas with comparable climates, including in the United States (e.g., the Maritimes, Newfoundland, eastern North Dakota to Maine).

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### Taphonomy, Postmortem Interval (PMI), Cold

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## H110 Smelling, Searching, and Sabotage: How Two Carrion Insects Compete for Decomposing Remains

Jennifer Rhinesmith-Carranza, BS\*, Texas A&M University, College Station, TX; Jeffery Tomberlin, PhD, Texas A&M University, College Station, TX

**Learning Overview:** This presentation will describe the findings from a suite of experiments conducted using *Lucilia sericata* blow flies and *Nicrophorus vespilloides* carrion beetles that explored their effects on volatile profiles of remains and their resource preferences. The results have potential implications for how the entomological pre-colonization interval for remains and its relationship with time of colonization estimation assumptions and Postmortem Interval (PMI) inferences are interpreted.

**Impact Statement:** This presentation will impact the forensic science community by highlighting some of the variability that exists in the entomological phases of decomposition, focusing on the pre-colonization interval. Attendees will better understand how insects of forensic importance locate and interact with remains and the variability associated with these interactions as related to estimating the time of colonization.

Resource location, evaluation, and acceptance by necrophagous insects are complex behaviors influenced by a combination of biotic and abiotic factors. How these factors govern resource-related behaviors of these insects is important in forensic science due to their influence on how Time Of Colonization (TOC) calculations as related to the PMI are determined. Human remains are a nutrient-dense resource pulse in the larger ecosystem that are utilized by insects. They are ephemeral, patchy resources, lasting between a few days and a few weeks or longer.<sup>1,2</sup>

Because of this variability and their nutritive value, there is intense competition among necrophagous insects for detection, location, and use.<sup>3</sup> Volatile Organic Compounds (VOCs) play an integral role in resource location by these insects and can also provide information about the quality and suitability of a resource conditional to insects' needs. Accordingly, it is crucial for insects to employ efficient mechanisms to process public information, such as volatile olfactory cues, in order to alter behavior (such as searching strategies) to maximize fitness.<sup>4</sup>

The common green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae), is the most common *Lucilia* species in the United States and, as a primary colonizer, its presence on remains can be used to determine TOC as related to PMI.<sup>5</sup> Similarly, the carrion burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae), has a palearctic distribution and is commonly found in cooler climates where they are considered a top competitor with these blow flies for small carrion.<sup>6</sup> Blow flies are known to "seed" their offspring's environment with commensal microbes, which, by nature, could alter the VOC profile of the remains, which could decrease competition with other species (i.e., change succession trajectory); *N. vespilloides*, moreover, show extensive carcass preparation that includes covering the resource in oral and anal secretions that contain a number of antimicrobial and antifungal compounds that could, similar to the blow fly, also alter the VOC profile and shift arthropod succession patterns.<sup>7-9</sup> As primary colonizers with significantly different resource use strategies, *L. sericata* and *N. vespilloides* provide an interesting model system in which to explore carrion use and effects on intra- and inter-specific competition and how these effects may influence colonization intervals and, thus, the relation of these intervals to PMI estimations.

This presentation will report results from a suite of experiments exploring the effects of: (1) *L. sericata* and *N. vespilloides* interaction with remains on the associated volatile profiles, (2) prior insect colonization and carcass age on adult *N. vespilloides* resource preference, and (3) prior insect colonization and carcass age on adult *L. sericata* resource preference in order to further our understanding of their detection, assessment, acceptance, and use of carrion and how that may influence the pre-colonization interval, which could have significant effects on how we view TOC estimations in forensic entomology.<sup>10</sup>

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### Entomology, TOC, Postmortem Interval (PMI)

## H111 Pre-Colonization Data Improves Larval Age Estimations With Human Remains

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**Learning Overview:** After attending this presentation, attendees will understand that even the best methods for estimating the time since death can be refined to increase accuracy. Attendees will learn about the first systematic attempt to quantify a previously understudied portion of the postmortem interval (the pre-Colonization Interval [pre-CI]) and how such data can be used to improve standard entomological time-since-death estimations.

**Impact Statement:** This presentation will impact the forensic science community by revealing a new method that can significantly decrease error in time-since-death estimations.

Forensic entomology currently represents one of the best approaches for estimating the minimum Postmortem Interval (PMI<sub>MIN</sub>) in medicolegal death investigations.<sup>1</sup> However, basic information regarding the period between death and insect colonization (i.e., the pre-CI) is lacking, and validation of standard estimation methods are rare<sup>2-7</sup>. Currently, no reference datasets for the pre-CI exists, though the inclusion of such data in a standard entomological estimation could improve accuracy.<sup>8</sup> The goals of this project were two-fold: (1) generate a baseline pre-CI dataset covering a range of ambient temperatures, and (2) examine the impact of using pre-CI data in conjunction with a larval age estimation on estimation accuracy. It was hypothesized that the addition of the pre-CI would increase estimation accuracy.

For the generation of the reference pre-CI dataset,  $N=62$  human donors were placed unclothed on the ground surface at the Anthropology Research Facility (ARF), University of Tennessee from February 2019 to July 2021. Observations for initial oviposition were made at least once a day. Accumulated Degree Hours (ADH) for the pre-CI were calculated from regional weather data.<sup>9</sup> Pre-CIs were grouped into six temperature bins: 0-10°C, 10.1-15.0°C, 15.1-20.0°C, 20.1-25.0°C, 25.1-30.0°C, and >30.0°C.

For larval age estimations,  $N=33$  human donors were sampled for blow fly larvae according to standard procedures, and blow fly species were identified using dichotomous keys.<sup>10-12</sup> Only larvae collected within 5,000 ADH of placement were used as accuracy decreased to 0% beyond this point. Estimations were made for each sampling timepoint, species, and development stage present in a sample. Two reference datasets per species (when possible) were used to generate estimations. The hypothesis that the estimated age was equal to the Time Of Placement (TOP) of each donor was tested. The average ambient temperature for the estimated exposure time was used to select the appropriate pre-CI for each sample. The mean pre-CI was then added to the maximum value of the estimated age range. An estimation was considered accurate if the true TOP was bracketed by the estimated range. Estimation accuracies before and after adding pre-CI data were analyzed via a non-parametric Wilcoxon Signed Rank Test.

The inclusion of temperature-specific pre-CI data in larval age estimations resulted in significantly higher estimation accuracy compared to those that excluded pre-CI data ( $P=0.002$ ). This translated to an 8% increase in overall accuracy (53% vs. 61%), with improvement of 21 underestimations from 13 donors. Improvements in estimation accuracy were not impeded by blow fly species, temperature, season, or any other environmental factors.

These results suggest that including temperature-specific pre-CI data to a standard larval age estimation decreases the likelihood of underestimating the true TOP, thereby increasing overall accuracy. This research represents the first attempt to systematically quantify the pre-CI with a large population of human remains over an extended period of time. This preliminary dataset also represents the largest effort to test and improve standard entomological estimation methods using human remains. Validation of the pre-CI dataset will continue with a larger population of human donors at the ARF.

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### Forensic Entomology, Postmortem Interval (PMI), Colonization

## H112 Successional Changes in the Entomological, Bacteriological, and Fungal Communities During the Decomposition of Swine Carcasses

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**Learning Overview:** Attendees will have the opportunity to learn about the communities of insects, bacteria, and fungi observed in different body regions of domestic pig (*Sus scrofa domesticus*) carcasses undergoing different stages of decomposition. Past work has established the succession of different groups of organisms during the process of decomposition of deceased organisms; this study intends to assess changes in the community assemblage of three different groups of decomposers (insects, bacteria, and fungi) and how each of these groups may influence each other. Attendees will also be able to learn how successional changes in decomposition can be applied to forensic investigations, specifically in the estimation of the minimum Postmortem Interval (mPMI).

**Impact Statement:** This presentation will enrich existing knowledge in the forensic science community regarding processes of decomposition, ecological succession, and their use in forensic investigations. The successional changes in entomological, bacteriological, and mycological communities noted during this study may provide reference information useful in forensic investigations involving a decomposing dead body.

One of the most common questions during forensic investigations is the estimation of the PMI, also known as the time of death.<sup>1</sup> In the past few decades, the application of forensic entomology has aided significantly in the estimation of the mPMI or Time of Colonization (TOC).<sup>2</sup>

Studies focused on the successional patterns of non-arthropod taxa on decomposing remains can also be helpful in forensic investigations; analyses of the microbial community and the fungal community present on decaying substrate during the different stages of decomposition have indeed shown a predictable pattern of species that can help the assessment of the PMI.<sup>3,4</sup> In this study, the successional changes of the entomological, bacteriological, and fungal communities during the decomposition of domestic pig (*Sus scrofa domesticus*) carcasses were investigated.

Twelve pig carcasses were exposed to the elements in a field in central New Jersey during the summer and allowed to decompose. Insects, bacterial swabs, and tissue samples for fungi were collected for each stage of decomposition. Fungi and bacteria communities were sampled from three different body regions (mouth, thorax/abdomen, and genitals/rectum) and were then identified using 16S rRNA and ITS gene sequencing, respectively. Immature insects (eggs and larvae) collected from the carcasses were reared to adulthood under lab conditions to morphologically identify the species using available dichotomous keys. The results of this study show changes in the community assemblages across the different stages of decomposition. The observed changes are in line with previous observations reported in relevant literature and appear related to the physical and biochemical changes occurring during decay. While the field of microbiological and fungal succession is still young and more research needs to be conducted before it can become truly applicable in forensic investigations, the results of this study do enrich our current knowledge of entomological, microbial, and fungal successional patterns during decomposition.

With growing datasets from studies of this type, this study intends to provide the forensic science community with additional data that can be used as reference in the estimation of mPMI in forensic investigations.

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### Decomposition, Succession, Postmortem Interval (PMI)



## H113 A Characterization of Volatiles Produced by Five Aerobic Postmortem Bacterial Species

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**Learning Overview:** After attending this presentation, attendees will be able to identify key volatiles produced by postmortem microorganisms and explain how volatiles develop over time.

**Impact Statement:** This presentation will impact the forensic community by linking key bacterial species to major components of decomposition odor. These chemical signals may act as key molecules for insect attraction, scent-detection canine alerts, or as targets for future sensor devices targeting decomposition odor.

Volatile Organic Compounds (VOCs) are compounds with an appreciable vapor pressure, and in combination can produce complex odor profiles. These compounds have vast probative potential because they are associated with several types of physical evidence, such as accelerants, drugs, currency, and decomposing remains. Postmortem VOCs are currently becoming of particular interest for possibilities in aiding medicolegal death investigations.

Postmortem VOCs may have great potential in the location of clandestine graves and estimation of postmortem interval. Postmortem microorganisms are also of great interest. They represent a major component of the postmortem food web because they contribute directly to the decomposition of remains and help to attract other decomposers. Individual VOCs can be attributed to postmortem microbial species studied in isolation according to recent studies. This experiment was conducted to characterize the VOCs released by *Curtobacterium luteum*, *Vagococcus lutrae*, *Bacillus subtilis*, *Ignatzschineria indica*, and *I. ureiclastica* using comprehensive Two-Dimensional Gas Chromatography-quadrupole Mass Spectrometry/Flame Ionization Detection (GC×GC-qMS/FID).

VOCs were collected from the headspace above each species incubated on standard nutrient agar in a laboratory setting and monitored over a period of five days. The objectives of this research were to provide longitudinal profiles for each individual microbial species and begin a database of microbial VOC data for comparison to future decomposition odor studies.

Of the data collected, hundreds of VOCs were detected. The standard nutrient agar emitted a complex VOC profile itself, requiring compounds of interest from bacteria to be identified by means of abundance comparison. A feature selection process was performed to identify peaks of interest for each species.

- 18 VOCs of interest were detected in *B. subtilis*, with a large number of aromatics, nitrogen-containing compounds, and halogenated compounds.
- 19 VOCs of interest were detected in *C. luteum*, with a nearly equal distribution across the detected compound classes.
- 21 VOCs of interest were detected in *V. lutrae*, with the fewest compound classes represented of all microbes tested. Aldehydes, esters, and ethers were absent from this species, whereas these were typically present for other species.
- 8 VOCs of interest were detected in *I. indica*, which were largely comprised of aromatic and polysulfide compounds.
- 25 VOCs of interest were detected in *I. ureiclastica*, which were largely contributed by aromatics, ketones, and alcohols.

Although the number of VOCs of interest identified after the feature selection reduced the number of compounds to around 20 compounds per species, the total number of unique compounds detected across the entire research study was 55. This count represents compounds that were differentiated from control samples of blank agar. The list of VOCs of interest provides insight into the origin of postmortem VOCs and typical temporal trends that may be observed given minimal species interaction and/or competition. The combination of numerous bacterial species exploiting a cadaveric resource can create an additive VOC profile that is more challenging to interpret in complex mixtures. The current study will serve to provide foundational data of the key VOCs contributed by each species, which has not been well understood in past decomposition odor research. This will also lay a foundation for future studies looking at VOC production when species are combined with one another to better understand how these bacterial species interact over cadaveric resources.

### Forensic Taphonomy, Postmortem Microbiology, Volatile Organic Compounds (VOCs)

## H114 Determining Human Identity From Leeches

*Veronica Cappas, BS\*, The Pennsylvania State University, University Park, PA; Reena Roy, PhD, The Pennsylvania State University, University Park, PA*

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**Learning Overview:** Attendees can expect to learn how human DNA profiles can be obtained from an annelid using direct amplification.

**Impact Statement:** The impact of this presentation will be manifested in the DNA analyst's ability to generate human DNA profiles from unlikely evidence such as an annelid using direct amplification.

Leeches are worms commonly found in water and can suck blood from a human host. These worms carry an anticoagulant, known as hirudin, in their salivary gland. These annelids have suckers, they conceal their cutting plates that hook onto human flesh, and after making a 2mm incision, they can then ingest from 5mL to 15mL blood from one human. If a perpetrator disposes of a victim's body in a river, stream, or pond, the leeches in the pool of water can hook onto the suspect's body. Once they are gorged with blood within about 30 minutes, they can fall off in the nearby area. In addition, they can help with identification of a victim or determine if a body had been in a certain area of the water. Therefore, they can become a valuable source of evidence for the forensic scientists.

The Roy research group has previously determined human identity from *Anopheles stephensi* mosquito blood meals using COPAN® microFLOQ® Direct swabs and PowerPlex® Fusion 6C System to identify Short Tandem Repeat (STR) DNA profiles. Massively parallel sequencing was also performed on the blood from the midgut of the same mosquitoes. In this current study, a similar approach was taken to identify human STR profiles from blood meal ingested by leeches. North American medicinal leeches were obtained from a commercial source, and blood from male and female donors were fed to individual leeches. After a certain period of time, each leech was frozen quickly, then dissected. Once blood was identified in the midgut with presumptive test, the presence of human blood was confirmed with various biological tests. After that, the tip of one swab was used to collect a minute amount of blood from the midgut area of each worm. These swabs, containing the human blood on their tips, were then used for direct amplification and STR profiles were determined using PowerPlex® Fusion 6C PCR amplification reagents. Known blood samples (reference samples) were also amplified using the COPAN® microFLOQ® Direct swabs and analyzed similarly. The Applied Biosystems™ 3130xl Genetic Analyzer was used for capillary electrophoresis. GeneMarker® HID V3.0.0 software was used for analyzing the STR profiles.

Complete and concordant profiles, consistent with the reference profiles of the donors of blood, were obtained from the midgut of the leeches using the above method. The results of this study indicate that COPAN® microFLOQ® Direct swab is an excellent way to amplify blood directly, even when the blood has been ingested by leeches. Complete STR profiles can be generated within a very short period using these swabs and the PowerPlex® Fusion 6C PCR amplification kit. Since only a minute quantity of blood is required, this method of collection and amplification is an excellent procedure for obtaining human identity from minute amounts of blood ingested by leeches and similar organisms. Research is being pursued to include sequencing of the blood from the leech midguts in order to obtain additional information about the human donors.

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### Direct Amplification, Human Blood, Leeches

## H115 2022 Jay Dix Day Lecture Series

*Michael Graham, MD, St. Louis University, St. Louis, MO and Office of the Medical Examiner, City of St. Louis, MO; Joseph Prahlow, MD\*, Western Michigan University, Homer Stryker M.D. School of Medicine, Kalamazoo, MI; Andrew Baker, MD\*, Hennepin County Medical Examiner's Office, Minneapolis, MN; Jonathan Hayes, MD\*, Office of the Chief Medical Examiner of the City of New York, New York City, NY; Joyce deJong, DO\*, Western Michigan University, Homer Stryker M.D. School of Medicine, Kalamazoo, MI; James Gill, MD\*, Connecticut Office of the Chief Medical Examiner, Farmington, CT*

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**Learning Overview:** Attendees can expect to learn how and why deaths related to previously specified topics occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

**Impact Statement:** Attendees will receive a comprehensive review of what causes and contributes to deaths related to the previously specified topics. They will be able to systematically evaluate deaths in which the previously specified topics may have played a role that they encounter in their daily practices.

Electricity is a ubiquitous entity in our lives. Some of it is intentionally generated to provide power and some of it originates as a force of nature (lightning). Interaction between humans and electricity is common and typically has no untoward effects. However, under some conditions this interaction may result in morbidity and/or mortality. Multiple causes, mechanisms, and contributory factors play a role in injury and deaths involving electricity. Understanding and evaluating injuries and deaths in which electricity may have played a role requires basic knowledge of electricity and how it affects various biological vital functions. Recognition of injuries and deaths caused by electricity is particularly important because of implications regarding the safety of others. This lecture will provide a comprehensive review of these issues.

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.

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.

There are multiple factors that can play a role in deaths that are temporally related to participating in and, occasionally, while 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 past three decades have seen an impressive expansion in the range of drugs available for abuse. The psychedelic revolution of the 60s spilled over into the 70s and 80s with the advent of "designer drugs" like Ecstasy (MDMA), joined in the 90s and 00s by the increased abuse of psychoactive drugs like ketamine and GHB, and, more recently, by the proliferation of powerful derivatives of the analgesic agent fentanyl and stimulants such as methamphetamine. A second pattern of expanded drug use involves increase in consumption in rural areas poorly served by the traditional distribution network for cocaine and heroin. The heartland of America has seen explosive growth in the use of methamphetamine, whether prescribed, homegrown, or cartel-distributed, and in diversion and abuse of prescription medication. Many of the drugs responsible for causing deaths are abused because of their sedating and/or stimulating properties. These substances are commonly used in characteristic scenarios and have somewhat stereotypical death scenarios. Recognition of the patterns of abuse of traditional and novel toxicants helps in assessing the role, if any, of these agents in particular deaths. This lecture will provide a comprehensive review of pharmaceutical and non-pharmaceutical toxicants in the context of investigating deaths.

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## Forensic Pathology, Pathology, Death Investigation

### H116 The National Center for Forensics—Forensic Workforce Development

*Danny Milner, MD, ASCP, Chicago, IL; Joseph Dizinno, DDS, George Mason University, Fairfax, VA; Anthony Falsetti, PhD, George Mason University, Fairfax, VA; Amie Ely, JD, NAAG, Washington, DC; Robert Kurtzman, DO\*, Montana State Medical Examiner's Office, Billings, MT*

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**Learning Overview:** After attending this presentation, attendees will understand the current barriers (real or perceived) for trainees to choose, successfully complete, and matriculate as forensic pathologists in the United States. Further, attendees will be able to explain multiple solutions/approaches to decreasing/removing these barriers to increase the forensic workforce.

**Impact Statement:** This presentation will impact the forensic science community by presenting work being conducted through the National Center for Forensics by the American Society of Clinical Pathology (ASCP) in collaboration with George Mason University on increasing forensic pathology workforce.

The National Center for Forensics was established through a competitive grant funded by the National Institute of Justice (NIJ) for the purpose of providing scientific and technical learning opportunities for the medicolegal community specifically targeted for medical students, and focused on underserved rural areas; providing forensic science and legal training, information, and support to law enforcement, district judges, and other appropriate criminal justice agencies; and developing, consolidating, and providing access to resources and opportunities for education, training, and best practices in the forensic science community, structured to benefit current and future practitioners in the field. This work includes an overall goal of increasing the pathology workforce through feasible, practical interventions.

The current forensic pathology workforce of approximately 500 board-certified forensic pathologists does not meet the current need in the United States of at least 1,200. This work conducted a literature and existing resources review, created structured questions across four areas, and completed nine focus groups using a diverse set of individuals from inside and outside of the practice of forensic pathology. Using the existing information and insights from focus groups, this work identified barriers in forensic pathology for medical students, residents, fellows, and practicing pathologists. These barriers include salary gaps, microaggressions, debt ratios, and workplace support/resources. This study hypothesized several potential solutions to combat these barriers that need to be tested for impact. From this work, working groups are to be formed with the intention of launching an additional global survey with the final results being feasible interventions focused on the specific barriers described.

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#### Forensic Pathology, Workforce, Focus Groups

## H117 The National Center for Forensics—The Medical Examiner’s Role

*Robert Kurtzman, DO\*, Montana Forensic Science Division Medical Examiner’s Office, Billings, MT; Dan Milner, MD, American Society for Clinical Pathology, Chicago, IL; Anthony Falsetti, PhD, George Mason University, Washington, DC; Joseph DiZinno, DDS, George Mason University, Washington, DC; Amie Ely, JD, National Association of Attorneys General, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will understand the medical examiner role with the National Center for Forensics and the concept of a coordinated state medical examiner’s office/county coroners function utilizing a “coroner liaison.”

**Impact Statement:** This presentation will impact the forensic science community by presenting work being conducted at the Montana State Medical Examiner’s Office (MTMEO) in coordination with the state’s 56 county coroner offices to promote consistent, timely, and cost-effective death investigation.

The National Center for Forensics was established through a competitive grant funded by the National Institute of Justice (NIJ) for the purpose of providing scientific and technical learning opportunities for the medicolegal community specifically targeted for medical students and focused on underserved rural areas; providing forensic science and legal training, information, and support to law enforcement, district judges, and other appropriate criminal justice agencies; and developing, consolidating, and providing access to resources and opportunities for education, training, and best practices in the forensic science community, structured to benefit current and future practitioners in the field.

Fifty-six county coroner offices investigate death in Montana which is the fourth-largest state in the United States and has a population of approximately 1,040,000. When a Board-Certified Forensic Pathologist (BCFP) is needed to perform a postmortem examination, coroners utilize the MTMEO. The MTMEO lost all state medical examiners in July 2015. Following restructure, the MTMEO was staffed with four full-time BCFPs, two full-time autopsy assistants, and two part-time autopsy assistants working in two regional offices as of April 2019. The concept of a “coroner liaison” was contemplated to facilitate collection of consistent, timely death investigation from coroners for the BCFPs with an online Medicolegal Death Investigation (MDI) reporting system known as the Montana Death Case Management System (MTDCMS). The system, first implemented in December 2019, includes an Application Platform Interface (API) with the state’s Vital Statistics Bureau for automated death certificate completion, purportedly the first such system operating in the United States.

The system, MTDCMS, is currently utilized by 39 of 56 county coroners with a desired goal of 100% utilization and is supported by recent legislation. With the NIJ grant, the concept of a full-time, American Board of Medicolegal Death Investigation (ABMDI) -certified coroner liaison came to fruition and was hired and began work August 2021. The addition of the coroner liaison has made an immediate impact to facilitate improved information collection; improve MTDCMS utilization by coroners and lessen the burden of information collection and MTDCMS promotion otherwise performed by BCFPs. The coroner liaison assists with development of forensic training with BCFPs for coroners and frees time dedicated to medical student training. As of this writing, two medical students have served a four-week rotation designed to promote interest in pursuing a career in Forensic Pathology (FP). Additional rotations have been scheduled. Thus far, students have expressed an interest in FP and long-term results will need to be tracked.

In summary, the MTMEO serves a large rural state in partnership with 56 county coroners with a small, cost-effective staff to promote uniform death investigation utilizing a state-wide online MDI reporting system facilitated with a coroner liaison. The MTMEO encourages and provides opportunities for medical students and stakeholders in forensic science to experience death investigation in a state system that serves mostly rural communities and better understand the challenges and opportunities that exist in that environment.

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### Coroner Liaison, Medical Examiner, Death Investigation

### **H118 Gunshot-Related Fatalities in the Pediatric Population: A Retrospective Study From the Cook County Medical Examiner's Office**

*Lorenzo Gitto, MD\*, State University of New York, Upstate Medical University, Department of Pathology, Syracuse, NY; Ponni Arunkumar, MD, Cook County Medical Examiner's Office, Chicago, IL*

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**Learning Overview:** After attending this presentation, attendees will better understand the prevalence and features of firearm-related deaths in the pediatric population.

**Impact Statement:** This presentation will impact the forensic science community by adding information about a topic rarely discussed in the current forensic literature.

Deaths due to gunshot wounds are commonly encountered by forensic pathologists worldwide. While the generic features of firearm fatalities are classically described in the forensic literature, only scant information can be found regarding pediatric deaths due to gunshot wounds. Although most deaths occur in young adults or adults, a minor population of young children is also affected. This study reports a series of pediatric deaths due to gunshot wounds from a large metropolitan area

The Cook County Medical Examiner's Office files were searched for deaths due to gunshot wounds between January 2016 and December 2020. This study considered the general definition of "pediatric population" as subjects between 0 and 18 years. The age was then subcategorized into multiple age groups. Only cases in which gunshot wounds were the primary and immediate cause of death and a clear and reliable manner of death was reported were included in the study. Delayed complications of gunshot wounds were excluded. Information regarding the cases was obtained from the investigative reports, the autopsy reports, and toxicological studies.

A total of 513 pediatric deaths due to gunshot wounds were found in the Cook County Medical Examiner's Office database. After excluding cases of delayed complication of gunshot wounds, 498 cases met the study criteria. The study population included 451 males and 47 females, with a mean age of 16 years; 378 subjects were African American, 95 were Latino, 24 were Caucasian, and 1 was Asian. The manner of death was homicide in 454 cases, suicide in 35 cases, undetermined in 7 cases, and accident in 2 cases. A single gunshot wound was observed in 259 cases, while multiple gunshot wounds were present in 239 cases; 405 deaths occurred in the city of Chicago, while the remaining 93 occurred in other cities.

This study will illustrate distinct features of pediatric deaths due to gunshot wounds. Compared to gunshot wounds deaths in adults, those involving the pediatric population may be more challenging. In this study, different age groups highlight the patterns of injury, the manner of death, circumstances of the event, and other aspects in this specific population. A thorough discussion of the results will be presented to attendees.

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**Forensic Science, Gunshot Wound, Pediatric**

### H119 Human Fatalities Caused By Dogs: A 17-Year Retrospective Study in Harris County, Texas, and New York City, New York

Hannah Jarvis MBBS, MRCS\*, Harris County Institute of Forensic Sciences, Houston, TX; Gregory Dickinson, MD, Office of Chief Medical Examiner of the City of New York, New York, NY

**Learning Overview:** After attending this presentation, attendees will gain an understanding of the circumstances, autopsy findings, toxicological results, and epidemiological patterns of human fatalities caused by dogs.

**Impact Statement:** This presentation will impact the forensic science community and public health communities by providing insight into human fatalities caused by dogs to promote public health safety campaigns and help forensic pathologists evaluate fatalities.

There are approximately 300,000 emergency room visits and up to 13,000 hospitalizations due to dog bite injuries every year in the United States. Dog bite-related fatalities are rare and account for approximately 18 deaths per year in the United States. Texas has one of highest incidence rates of dog bite-related fatalities in the United States. Most of these deaths are in children, occur on the owner's property, by the family's own dog, and involve pit bull-type dogs.<sup>1</sup>

This study reviews the case files of all fatalities related to injuries caused by dogs over a 17-year period in Harris County, TX, and New York City, NY, to study the circumstances, autopsy findings, toxicological results, and epidemiological patterns of these fatalities.

The electronic databases of the Harris County Institute of Forensic Sciences and Office of Chief medical Examiner of the City of New York were searched for all fatalities related to dogs between 2003 and 2021.

Of the 23 fatalities, 13 were male and 10 were female, and the average age was 37.5 years, ranging from 16 days to 80 years old, with seven cases being 4 years of age or younger. Most were White ( $n=10$ ) and three were homeless people. Half of the injuries ( $n=12$ ) occurred within a residence. Where known, the dogs involved in the deaths were mostly pit bull-type dogs ( $n=6$ ). The most frequent location where the attacks occurred were Houston, TX, ( $n=6$ ) and the borough of Brooklyn in New York City, NY ( $n=3$ ). Attacks frequently occurred in the winter ( $n=6$ ); and between 4:00 p.m. and 5:00 p.m. ( $n=3$ ) or 9:00 p.m. to 10:00 p.m. ( $n=3$ ). Toxicological analysis revealed illicit substances (cocaine and methamphetamine) and ethanol in six cases.

Eighteen cases underwent autopsy examination and five cases underwent external examination only. The most commonly injured body regions were the extremities ( $n=17$ ) and head ( $n=14$ ). Some of the notable injuries included skull fractures (open and closed), cervical spine fracture with spinal cord injury, other fractures (forearm and ribs), vascular injuries (radial and carotid artery, internal jugular vein), crush injury (larynx), and visceral lacerations (lung, heart, liver). Deaths with no survival interval ( $n=12$ ) were due to multiple blunt and sharp force injuries caused by dog bites or a sudden cardiac arrhythmia during the attack ( $n=1$ ); and those with a survival interval ( $n=10$ ; range 2 days to 19 years) were due to infective complications, including one case of rabies meningoencephalitis, or pulmonary thromboemboli complicating remote injuries of the lower extremities. Twenty-one cases were certified as Accident as the manner of death; one case was certified as Natural (rabies meningoencephalitis); and one as Homicide (neonate).

It is important for a forensic pathologist to be able to identify these injuries as being caused by a dog and not criminal assault and to recognize the patterns. Studies show the rate of deaths from injuries due to dogs is increasing, and are seen frequently in children.<sup>1</sup> Most dog owners are not aware of factors that increase the risk of dog bites, and public safety campaigns should be promoted. A collaborative and multidisciplinary approach is necessary to try to prevent these fatalities.

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#### Dog Bite, Pit Bull, Injury to Children

## H120 Homeless Mortality Reporting by the King County, Washington, Medical Examiner's Office: Impact and Importance

Nicole Croom, MD, MPH\*, King County Medical Examiner's Office, Seattle, WA; Richard Harruff, MD, PhD, King County Medical Examiner's Office, Seattle, WA

**Learning Overview:** After attending this presentation, attendees will be able to describe homeless mortality reporting by the King County Medical Examiner's Office (KCMEO) and how it serves public health and the affected community.

**Impact Statement:** Recognizing that homelessness is a national crisis, this presentation shows how death investigation systems can provide unique data informing and evaluating community responses.

**Introduction:** On any given night in the United States, there are roughly 567,715 Individuals Experiencing Homelessness (IEH).<sup>1</sup> The number of IEH has decreased by 12% overall since nationwide data collection began in 2007 but has been steadily rising since 2018, as measured by Point-in-Time Counts (PITC), indicating that recent events have reversed progress in eliminating homelessness.<sup>1</sup> Federal health statistics do not count the number of IEH deaths.<sup>1</sup> To fill this gap, the non-profit National Health Care for the Homeless Council began monitoring IEH mortality in 2019.<sup>1</sup> Unfortunately, only 2% of United States counties report decedent housing status.<sup>2,3</sup> Extrapolating data from reporting cities to the national PITC, the Council estimates that between 17,500 and 46,500 IEH deaths occurred in 2018, though this may be an underestimate due to non-standardized definitions of housing status.<sup>2,3</sup>

KCMEO serves a population of about 2.3 million of which 11,751 are IEH as estimated by the 2020 PITC; a 5% increase in homelessness compared to the 2019 PITC.<sup>4,5</sup> Since 2010, KCMEO has compiled homeless mortality data and shares this information with community stakeholders. This presentation reviews KCMEO's homeless death reporting, analyzes IEH mortality in King County from 2010-2020, evaluates how this data serves community responses in combating the homelessness crisis, and encourages other death investigation offices to participate in standardized data collection measuring IEH mortality.

**Methods and Materials:** During the period of this study, 2010–2020, medicolegal death investigators recorded housing status for jurisdictional deaths using standardized criteria developed in conjunction with Public Health–Seattle and King County, Healthcare for the Homeless program. The data were entered into KCMEO's VertiQ database along with demographics, death certificate information, and narrative accounts describing each death. Quantitative data for this presentation were analyzed using Microsoft® Excel®. Qualitative data came from interviews with community stakeholders who use homeless mortality data.

**Results:** Between 2010 and 2020, KCMEO assumed jurisdiction on a total of 27,583 cases, with an overall annual increase of 87% over this period. Over this period, there were 1,346 deaths of IEH; the proportion of IEH death out of the total varied from 2.2% to 7.6%, with the maximum in 2018. Of IEH deaths, the average age was 49 years, 19% were female, and 74% were given the racial categorization of White. Manners of death were Accident in 46%, Homicide in 4.5%, Natural in 37%, Suicide in 6.8%, and Undetermined in 5.5%. Acute drug intoxications accounted for 80% of those certified Accident and 37% of total IEH deaths. Cardiovascular disease was the most common cause (36%) of IEH deaths certified Natural. Interviews found that community stakeholders use mortality data for a variety of purposes, including health care coordination and management, evaluation of outreach efficacy, next-of-kin notification, and to “raise up the dignity of the dead.”

**Conclusion:** This study demonstrated important differences in mortality among the homeless compared to the general population investigated by KCMEO. These data represent an important metric for public health and community groups responding to the homelessness crisis. Considering that only 2% of counties report mortality data for IEH, death investigation systems have an opportunity to participate in the response by collecting and reporting housing status data of decedents.

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### Individuals Experiencing Homelessness, Mortality Review, Program Evaluation



### H121 An Analysis of Two Decades of Intimate Partner Femicide-Suicide in Turkey

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**Learning Overview:** Attendees of this presentation will learn about the term murder-suicide, the increasing rates of Intimate Partner Female Murder-Suicide (IPF-S) incidents in the media news broadcast in Turkey, and the characteristics of the victims and perpetrators of the incidents.

**Impact Statement:** This presentation will impact the forensic science community by identifying the methods that perpetrators use to kill and commit suicide, the characteristics of the victims, and the perpetrators of the incidents.

IPF-S is an understudied subgroup of homicide-suicide deaths. There is limited research handling IPF-Ss in the Eastern Mediterranean region. This study aimed to evaluate the characteristics of IPF-Ss that occurred in Turkey between 2000 and 2019. The IPF-Ss ( $n=226$ ) were extracted from electronic news stories. This study collected data about victims, perpetrators, their relationships, and the incidents of murders and suicides. Descriptive statistics were calculated, and logistic regression analysis, mortality rates, and the proportion of IPF-S in femicides calculation were conducted, showing that 13.3% of the femicides ( $n=1,699$ ) were IPF-Ss. The IPF-Ss were increasing in five-year intervals, which were highest in the 2015–2019 period (62.5%). The victims were married in 48.2% of the cases, 56% were younger than 35; while the perpetrators were married in 51.3%, and older than 40 in 52.6% of the cases. In 42.0% of the cases, the perpetrators were living together with the victim. Most (79.2%) of the cases were in urban settlements, and perpetrators used firearms in 84.1% of the femicides. Firearms were a more common method in cases where IPF-Ss were planned (OR=2.98), when the IPF-S method was the same (OR=29.6), and when the perpetrator committed suicide (OR=7.82).

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#### Homicide/Suicide, Femicide, Violence Against Women

## H122 Influencing Factors on Postmortem Protein Degradation for Postmortem Interval (PMI) Estimation: A Systematic Review

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**Learning Overview:** This presentation provides an overview of the research on influencing factors on forensic postmortem protein degradation, discusses limitations and the current state of knowledge, and addresses future perspectives and important open research questions.

**Impact Statement:** Estimation of the PMI is of critical importance in forensic routine, but often extremely difficult. Novel techniques to contribute to the current methodic spectrum are certainly required, but information on influencing factors is scarce. This presentation highlights the immense importance of this issue for PMI estimation and provides the basis for targeted future research.

**Background:** A most precise estimation of the PMI is a critical and yet extremely difficult task in forensic routine. In the past decades, biochemical analysis of tissue decomposition, especially protein degradation, has become of significant interest in this regard.

While there is evidence for the breakdown of specific proteins in a number of different tissues, little is published on (possible) individual and environmental influencing factors, despite the striking importance of this issue for the application in casework. This literature review aims to investigate the current state of knowledge and the future perspectives of influencing factors on postmortem protein decomposition for the use in forensic PMI estimation.

**Objectives:** This study provides a systematic review of the literature to evaluate the current research status of influencing factors on protein degradation-based PMI estimation. Focus was set on characteristics of internal and external influencing factors and the respective susceptibility and/or robustness of protein degradation.

**Method:** A systematic literature search up to December 2020 was conducted on the effect of influencing factors investigated in the context of postmortem protein degradation in the tissues of animals and humans using the scientific databases PubMed® and Google® Scholar, as well as the reference lists of eligible articles.

**Results:** This work identified ten studies investigating a total of seven different influencing factors in degrading tissues/organs of humans and animals using six different methodological approaches. Although comparison of study outcomes was hindered by the high variety of investigated factors, and by high risk of bias appraisals, it was evident that the majority of the influencing factors concerned affected protein degradation, thus being able to modulate the precision of protein degradation-based PMI estimation.

**Conclusion:** The results clearly highlight the need for a thorough screening for corresponding factors to enable the introduction of appropriate correction factors and exclusion criteria. This seems especially relevant for the protein degradation-based study of human PMI to increase the reliability and precision of the method and to facilitate a broader applicability in routine forensic case work.

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### PMI Estimation, Protein Degradation, Influencing Factors

### H123 Postmortem Computed Tomography (PMCT) in Unwitnessed Choking: A Case Report and Retrospective Review of Seven Cases of Airway Obstruction

*Michael Harrell, MD\*, Office of the Medical Investigator, Albuquerque, NM; Lauren Decker MD, Office of the Medical Investigator, Albuquerque, NM*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the utilization of Postmortem Computed Tomography (PMCT) in triaging witnessed and unwitnessed (suspected) choking deaths.

**Impact Statement:** This presentation will impact the forensic science community by correlating findings from multiple autopsies with PMCT where the cause of death is choking. The aim is to improve the autopsy triage process by utilizing PMCT findings to determine the most appropriate examination type.

Choking is broadly defined as a form of asphyxia in which the internal airways are blocked; the manner of these deaths is most commonly classified as accident. Choking-related deaths may prove to be a diagnostic dilemma when triaging for autopsy. At the Office of the Medical Investigator, triage of suspected choking relies on scene evidence to guide the type of examination. Generally, witnessed chokings, including bystander or first responder testimony of the event, require an external examination. If the event is not witnessed, an autopsy is performed to confirm the presence of an obstructing bolus and rule out an alternative cause of death.

PMCT has proven invaluable in evaluating the pharynx and airways for the presence of foreign material.<sup>1,2</sup> For example, a 54-year-old man with a history of unspecified intellectual disability, hypertension, diabetes mellitus type 2, and remote aspiration pneumonia was found unresponsive in the living room of his group home. Emergency medical services were contacted, and no resuscitative efforts were performed given obvious signs of demise.

PMCT demonstrated abundant obstructive isoattenuating and hypoattenuating foreign material within the oropharynx, hypopharynx, proximal esophagus, trachea, and in the right and left mainstem bronchi immediately distal to the carina. Autopsy revealed the body of an adult male with no significant injuries. A large food bolus completely obstructed the hypopharynx, with abundant smaller food particles throughout the proximal airways, consistent with findings on PMCT. Other natural disease included cardiomegaly, atherosclerotic disease, nephrosclerosis, and steatosis. The cause and manner of death were certified as choking, accident.

A retrospective review of unwitnessed, adult choking deaths with findings at autopsy that underwent PMCT was performed at the Office of the Medical Investigator, in Albuquerque, NM, from 2018 to 2021. This review revealed seven cases, including the aforementioned case. All seven cases had PMCT findings that correlated with gross findings at autopsy. Six of the seven cases had large, obstructing foreign material in the hypopharynx, identified both grossly and by PMCT. One case had only distal airway foreign material that was less readily identified on PMCT. All cases had underlying conditions that predisposed to choking, including intellectual disability, dementia, substance intoxication, intracranial masses, remote stroke, and edentulousness.

Given the gross and PMCT correlations in this review and the referenced material, imaging should be considered informative in decision making regarding autopsy triage of witnessed and unwitnessed choking deaths. This may be advantageous for cultural and religious groups requesting minimally invasive examinations, or those with a marked food burden by PMCT as well as a condition that predisposed to choking. Further investigations would include correlative studies with larger sample sizes, especially involving children and decedents with decomposition changes.

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#### Autopsy, Choking, Accident

### H124 The Status of Internal Neck Structures in Violent Compression of the Neck: A Ten-Year Autopsy Study

*Nilesh Tumram, MD\*, Government Medical College, Chandrapur, Maharashtra, India*

**Learning Overview:** After attending this presentation, attendees will be able to recognize the status of internal neck structures in violent compression of the neck, particularly the damage caused by it.

**Impact Statement:** This presentation will impact the forensic science community by creating understanding of damages caused by violent compression of the neck. Autopsy findings such as contusion, tears, and hemorrhages in internal neck structures like oropharynx, laryngopharynx, and larynx in cases of fatal compression of neck can be of important medicolegal significance. Presented is such a study that highlights its occurrence and its possible significance to correlate with such incidences.

Fatal compression of the neck due to strangulation, hanging, throttling, etc. are typical forms of suicidal, accidental, and homicidal deaths. External injuries such as ligature mark, periligature injuries, abrasion, contusion over the neck, and internal injuries like hyoid bone fracture, thyroid cartilage fracture, neck muscle hematoma, etc. are utilized to opine regarding the antemortem nature of fatal compression of the neck. However, sometimes the above findings may be minimally present. In such cases, some other findings occurring due to compression of the neck can also be very useful and additive for opining in such incidences.<sup>1,2</sup>

Out of total 30,310 autopsy cases occurring in a period of ten years at the autopsy center, this work has studied 280 cases of violent compressions of the neck. Apart from the autopsy findings such as ligature mark over the neck, dribbling of saliva, hemorrhages over the neck muscles, fractures of the hyoid and thyroid cartilage, injuries occurring in the internal neck structures like the oropharynx, laryngopharynx, and larynx were studied. Such findings can be useful for commenting on the antemortem nature of such cases together with the other established findings in such cases. Also, such findings can become significant if there are minimal or no findings available in cases of fatal compression of the neck.

In postmortem examination of victims of hanging, ligature strangulation, and throttling who exhibited relatively sparse external findings of significance, a unique and apparently less-described patterned hemorrhage within the oropharynx, laryngopharynx, and larynx can become crucial. In cases where strangulation is suspected as well as other potentially important violent neck compressions, a detailed inspection of these structures may provide invaluable information.

**Reference(s):**

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2. Bockholdt, B.; Maxeiner, H. Hemorrhages of the tongue in the postmortem diagnostics of strangulation. *Forensic Sci Int.* 2002 May 23; 126(3):214-20.

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**Violent Compression of the Neck, Internal Neck Structures, Autopsy**

### **H125 A Comparison of Findings Identified at Traditional Invasive Autopsy and Postmortem Computed Tomography (PMCT) in Suicidal Hangings**

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**Learning Overview:** Following this presentation, attendees will have a better understanding of the efficacy of PMCT to detect pertinent findings in cases of suspected suicidal hanging.

**Impact Statement:** This presentation provides an evidence base for the use of PMCT in cases of suicidal hanging, which is a common case type referred for medicolegal autopsy throughout the world.

The reports of the invasive autopsy and PMCT images were reviewed in 50 cases of suspected suicidal hanging referred to the State Pathologist's Department, Belfast, during an 11-month period. The Traditional Invasive Autopsy (TIA) examinations included an external examination and full internal examination completed by a consultant forensic pathologist. The PMCT images, which included the head, neck, chest, abdomen and pelvis, were reported by a consultant radiologist. The pathologist and radiologist were both blinded to the findings of the other modality. A Cohen's Kappa coefficient (K) was subsequently calculated to assess the agreement between the TIA and PMCT across a range of pertinent findings. This demonstrated perfect agreement with the identification of a ligature (K=1.00) and a strong level of agreement for the identification of a ligature suspension point (K=0.832). However, there was only a minimal level of agreement for the overall ligature mark (K=0.223). In addition, PMCT demonstrated a weak level of agreement for fractures of hyoid bone (K=0.555) and thyroid cartilage (K=0.538), but the invasive autopsy was shown to not be infallible as three probable fractures were identified on PMCT only. Furthermore, the TIA was shown to be superior in the identification of intramuscular and laryngeal fracture-related hemorrhage/bruising. In contrast, PMCT was superior to TIA in the identification of gas deposition within the body, including subcutaneous emphysema and fracture-associated gas bubbles. However, this study would question the usefulness of these findings, particularly in decomposed bodies. Finally, although not a primary study objective, there was overall good correlation between the natural disease and trauma identified elsewhere in the body during the TIA and PMCT, with only a few minor discrepancies reported.

The study demonstrated that PMCT can assist the investigation of suspected suicidal hangings, but the accuracy of many findings is limited, and the technique should not be used in the absence of, at the very least, a detailed external examination completed by an experienced autopsy pathologist.

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#### **Hanging, PMCT, Autopsy**

## **H126 An Autopsy Study of Anthropometric Measurements and Indices of Obesity and Their Relationship With Coronary Artery Atherosclerosis in a South Indian Population**

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**Learning Overview:** After attending this presentation, attendees will appreciate the relationship between anthropometric obesity measurements and coronary atherosclerosis; gain a better understanding of gender differences with respect to these measurements and indices; and find out if these measurements are coronary artery specific.

**Impact Statement:** The impact of this study lies in its findings that anthropometric measurements/indices of obesity can be used to identify high risk cases of atherosclerosis at an early stage. Early detection and appropriate preventive or corrective measures can then be instituted to reduce the associated cardiac morbidity and mortality.

The association between human physique and morbidity and mortality resulting from coronary artery disease has been studied extensively over several decades. Multiple studies have also been done on the correlation between grade of atherosclerosis, coronary artery diseases, and anthropometrical measurements.<sup>1-4</sup> However, the number of autopsy-based studies drastically reduces this number. While in living subjects, it would be expensive, difficult, and even harmful to subject them to imaging modalities like Computed Tomography (CT) scans and procedures involving contrast media to study mild atherosclerosis, no such harm is encountered in autopsied cases.<sup>5</sup>

This autopsy-based study was aimed to correlate the anthropometric measurements and indices of obesity such as Waist Circumference (WC), Hip Circumference (HC), Body Mass Index (BMI), and Waist Hip Ratio (WHR) with the degree of atherosclerosis in the Right Coronary Artery (RCA), main branch of the Left Coronary Artery (LCA), and the Left Anterior Descending Artery (LADA) in 95 South Indian origin victims of both sexes between the ages of 18 and 75 years. The grading of atherosclerosis was done according to criteria suggested by the American Heart Association. The study also analyzed the correlation of the anthropometric measurements and indices of obesity with the number of coronaries affected.

All the anthropometric measurements and the derived indices were found to be significantly correlated to each other in both the sexes except for the age, which is found to have a significant correlation only with the WHR. In both the genders, a severe degree of atherosclerosis was commonly observed in LADA, followed by LCA and RCA. The grade of atherosclerosis in RCA is significantly related to the WHR in males. The grade of atherosclerosis in LCA and LADA is significantly related to the WHR in females. Significant relation was observed between the grade of atherosclerosis in RCA and WC and WHR, and between the grade of atherosclerosis in LADA and HC in males. Significant relation was observed between the grade of atherosclerosis in RCA and WC and WHR, and between the grade of atherosclerosis in LADA and HC in females.

Based on these findings, people with anthropometric measurements suggestive of mild atherosclerosis can be advised to modify their lifestyles, along with decreasing their exposure to other risk factors. This study also further underscores the importance of forensic medicine and autopsies as the best means of obtaining information related to cardiac changes.

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### **Atherosclerosis, Obesity, Coronaries**

## H127 A Tale of Unrelenting Chest Pain: The “Short” Versus the “Tall”

Robyn Parks, MD\*, Los Angeles County Department of Medical Examiner-Coroner, Los Angeles, CA

**Learning Overview:** After attending this presentation, attendees will be reminded of the importance of completing genetic variant testing in thoracic aortic dissections to detect underlying congenital connective tissue disorders that may impact surviving family members.

**Impact Statement:** This presentation will impact the forensic science community by presenting two cases of ruptured thoracic aortic dissections with underlying, previously unknown, congenital connective tissue disorders and serve as a reminder of the importance of genetic variant testing and close family communication.

A 38-year-old man without diagnosed medical conditions or illicit drug use complained of chest and back pain. He visited an urgent care and was advised to follow-up at an emergency room, but never sought additional care. One week later he was found unresponsive in the bathroom by his roommate, who subsequently called 9-1-1.

Paramedics arrived and pronounced death at the scene. Autopsy revealed a 74-inch, 154-pound man (body mass index 19.8kg/m<sup>2</sup>) with an arm span of 77 inches (height/arm span ratio: 1.04), and positive thumb sign. Internal examination revealed hemopericardium (600cc), left greater than right hemothorax (400cc versus 25cc), large hematoma surrounding the proximal aorta, and aortic root dilatation (5.5cm at the sinus of Valsalva, aortic root Z-score 8.06).<sup>1</sup> Cardiac examination revealed a nearly circumferential intimal tear of the ascending aorta with an associated full thickness defect, 1.5cm distal to the aortic valve without aortic valve involvement. On the day of autopsy examination, the findings, diagnosis, and potential for an underlying genetic condition were explained to the family. The Aortopathy and Connective Tissue Disorder Panel was ordered through Invitae Corporation and returned with a heterozygous pathogenic variant in FBN1, which is associated with autosomal dominant Marfan syndrome and related conditions. These results were immediately relayed to family members and the potential for first-degree relatives to receive free testing for this variant was explained. The cause of death was certified as cardiac tamponade due to ruptured thoracic aortic dissection due to Marfan syndrome (FBN1 heterozygous pathogenic variant), and the manner of death listed as natural.

A 29-year-old man without diagnosed medical conditions, illicit drug use, or alcohol use, who was known to not seek out routine medical care, was awoken from sleep with chest and mid-upper back pain. He drove himself to the emergency department where he was diagnosed with kidney failure and possible pleuritic pain, prescribed prednisone, and discharged. His symptoms persisted and, three days later, his father heard a “thud” from his son’s bedroom, where he found him on the floor. 9-1-1 was called and he was instructed to perform lay-person cardiopulmonary resuscitation until paramedics arrived, at which time death was pronounced at the scene. Autopsy revealed a 66-inch, 211-pound man (body mass index 34.1kg/m<sup>2</sup>) without external trauma. Internal examination revealed hemopericardium (800cc) with an aortic dissection coursing from the proximal ascending aorta with right coronary artery ostium involvement, distally along the length of the aorta and involved bilateral common iliac arteries. Additionally, the organs were extremely friable with extensive lacerations of the liver attributed to bystander cardiopulmonary resuscitation efforts. Similar discussions with family occurred, and the same panel was sent to Invitae. A heterozygous-likely pathogenic variant in COL3A1 associated with autosomal dominant Ehler-Danlos syndrome was detected. The cause of death was certified as cardiac tamponade due to ruptured thoracic aortic dissection due to congenital connective tissue disorder (heterozygous COL3A1 likely pathogenic gene variant).

These cases highlight the spectrum of connective tissue disorders and the importance of medical examiners/coroners pursuing genetic variant testing, along with emphasizing close family communication to ensure appropriate clinical follow-up for surviving family members.

### Resource(s):

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### Aortic Dissection, Connective Tissue Disorder, Marfan Syndrome

## H128 Restraint-Related Asphyxia, Excited Delirium, and Drugs: The Role of Counterfactual Causation in Assessing the Cause of Death in Custody

Michael Freeman, PhD, Maastricht University, Maastricht, Limburg, Netherlands and Oregon Health & Science University School of Medicine, Portland, OR and Faculty of Forensic and Legal Medicine, Royal College of Physicians, London, England, United Kingdom; Ellen Strommer, MPH\*, Maastricht University, Maastricht, Limburg, Netherlands

**Learning Overview:** The goal of this presentation is to describe a framework for systematic analysis of deaths in custody, when both intoxicants and restraint are present, in the context of evolving research.

**Impact Statement:** This presentation will impact the forensic science community by presenting an evidence-based format for assessing the role of restraint in causing an in-custody death. This will be accomplished by first describing a counterfactual framework for evaluation of evidence, in which the pivotal question is what was the risk of death in the absence of the restraint? Next, the evidence of risk associated with all plausible and substantive competing causes is quantified and compared as a ratio (i.e., restraint versus all other putative causes). The result can be presented in the form of a ratio of risks that can control for the potential role of cognitive bias in the assessment.

In custody deaths that occur in the context of restraint or the use of force present unique difficulties for the investigation of manner and cause of death. This is particularly true because the mechanism by which restraint can cause death is typically asphyxia, and the amount of force or restraint necessary to result in sufficient asphyxia in an individual to trigger a cardiopulmonary arrest is widely varied and dependent on the physiologic respiratory requirements of the individual prior to the initiation of the restraint, the nature and duration of the restraint, and the physiognomy and cardiorespiratory fragility of the restrained subject.

There is a body of literature that exculpates restraint as a cause of asphyxia and sudden death. This literature supports a complementary theory, which depends on the dual supposition that the death can occur spontaneously due to Excited Delirium (ExDS), a condition that leaves no evidence at autopsy, and at the same time rejects restraint-related asphyxia as a plausible cause of death due to the results of experiments performed on volunteers.

A recent review of the world literature on ExDS, as well as the literature describing simulated restraint experiments, has not only cast doubt on the validity of the ExDS diagnosis, as well as the scientific quality and generalizability of the experiments to the investigation of any real-world death, but demonstrated a direct dose-response between the nature (severity) of the restraint and the likelihood that a diagnosis of ExDS will be fatal (odds of death are 7.4 times greater when manhandling occurs, 10.7 times greater when the individual is handcuffed [and prone], and 50 times greater if the prone person was also hog- or hobble-tied).<sup>1</sup> These findings indicate that ExDS has been used historically to describe deaths when aggressive restraint has been used, which has had the (intentional or unintentional) effect of exculpating the restraint, as well as the individuals responsible for the restraint, as the cause of death.

There are currently no methods of quantifying the effects of restraint, in terms of asphyxial or cardiopulmonary arrest risk, in any given decedent after the effect. In part, this is due to the fact that by the time an agitated or delirious individual is restrained, their oxygen needs are already substantially increased over a resting state, and thus a lesser degree of restraint is required to result in physiologically meaningful hypoxia or asphyxia.

Counterfactual reasoning is a technique for evaluating the “but-for” probabilities within a causal investigation. In a death investigation, the approach asks the question, “Had the investigated adverse exposure not occurred, what is the likelihood the decedent would have died at the same point in time?” In the context of a restraint-related death, the cumulative risk of death from non-restraint-related explanations, including the presence of illicit drugs, alcohol, coronary artery disease, anomalous cardiac vasculature, i.a., can be estimated using methods and data forensic epidemiologic methods and data.

The risks can be quantified using available epidemiologic and other data, yielding an estimate of the death risk to the decedent in the absence of the restraint. This presentation will describe methods for obtaining such risks and comparing them to the risks associated with various means and duration of restraint, so that the resulting ratio of restraint risk versus all other risks can be estimated to provide a semiquantitative conclusion regarding the strength of a causal association between the restraint and the death in a specific investigation.

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### Death in Custody, Counterfactual Causation, Restraint-Related Asphyxia



### H129 Prone Restraint Cardiac Arrest: A Comprehensive Review of the Scientific Literature and an Explanation of the Physiology

Alon Steinberg, MD\*, Community Memorial Hospital/Western University, Ventura, CA

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**Learning Overview:** There is current controversy regarding the role of positional asphyxia as the primary factor for restraint-associated mortality. Some authors state that prone restraint position has no significant physiologic effects and that in prospective epidemiological studies, prone position has been shown to be safe. Other authors have described significant physiologic effects and retrospective epidemiological studies have shown that occurrence of death in prone position is not uncommon.

**Impact Statement:** This presentation will review scientific literature articles on the impact of prone restraint on respiratory physiology and cardiac output and will learn why a certain population of agitated subjects are at risk of death from prone restraint.<sup>1</sup> This is an important topic to understand as, ultimately, it is the forensic scientist who determines the cause of death in an often litigious case. This presentation will further educate the forensic science community regarding the possible causes of death in prone restraint. This presentation will lead to awareness, important discussion/debate, and further future study.

Deaths occurring among agitated or violent individuals subjected to physical restraint have been attributed to positional asphyxia. Restraint in the prone position has been shown to alter respiratory and cardiac physiology, although this is thought not to be to the degree that would cause asphyxia in a healthy, adult individual. This review identifies and summarizes the current scientific literature on prone position and restraint, including experiments that assess physiology on individuals restrained in a prone position. Some of these experimental approaches have attempted to replicate situations in which prone restraint would be used. Overall, most findings revealed that individuals subjected to physical prone restraint experienced a decrease in ventilation and/or cardiac output in prone restraint.

Metabolic acidosis is noted with increased physical activity, in restraint-associated cardiac arrest and simulated encounters. A decrease in ventilation and cardiac output can significantly worsen acidosis and hemodynamics. Given these findings, deaths associated with prone physical restraint are not the direct result of asphyxia but are due to cardiac arrest secondary to metabolic acidosis compounded by inadequate ventilation and reduced cardiac output. As such, the cause of death in these circumstances would be more aptly referred to as “prone restraint cardiac arrest” as opposed to “restraint asphyxia “or positional asphyxia.”

**Reference(s):**

- <sup>1</sup> Steinberg, A. Prone restraint cardiac arrest: A comprehensive review of the scientific literature and an explanation of the physiology. *Med Sci Law*. 2021:25802420988370.

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#### Police Custody Death, Restraint Physiology, Positional Asphyxia

## H130 Prone Restraint Cardiac Arrest

Victor Weedn, MD, JD\*, Office of the Chief Medical Examiner, Baltimore, MD; Alon Steinberg, MD, Cardiolog Associates Medical Group, Inc., Centura, CA; Pete Speth, MD; Wenonah, NJ

**Learning Overview:** After attending this presentation, attendees will understand the physiology of prone restraint cardiac arrests.

**Impact Statement:** The forensic pathology community should be aware of prone restraint as a cause of death in in-custody deaths.

The National Association of Medical Examiners (NAME) has promulgated a position paper on Deaths in Custody, but does not discuss causes of death.<sup>1</sup> Much of the literature has focused on mechanical asphyxia or excited delirium as the cause of these deaths.<sup>2</sup> Vilke et al. have claimed that prone restraint is not dangerous, but more recently, Steinberg has refuted this claim.<sup>3-5</sup> Presented are two cases that demonstrate the danger of prone restraint.

The first case involved an obese 51-year-old male subject (BMI 39.7). The subject used drugs (PCP/TCP, cocaine, and methadone), began “acting weird,” and cut his hand when he broke a ceramic plate on the kitchen counter. This woke up his brother-in-law, who then controlled him. When the police arrived, the brother-in-law was standing over the subject. The police quickly handcuffed the subject with his hands behind his back and shackled his legs, turned him on his side, and attempted to calm him. The subject continued to writhe and yell. Emergency Medical Services (EMS) was called and upon arrival, strapped him face down to a spine board, then placed him on a wheeled stretcher with the placement of further straps and loaded him into the ambulance. He became unresponsive during this time or immediately thereafter. He was turned supine and Advanced Cardiac Life Support (ACLS) measures were begun. He was found to be in bradycardia, then asystole, and later Pulseless Electrical Activity (PEA). He was transported to the hospital and was successfully resuscitated, but he never regained consciousness and was declared brain dead a week later.

Autopsy revealed an enlarged heart (650gms) and encephalomalacia. Cardiovascular consultation revealed hypertrophy, biventricular dilatation, and circumferential left ventricular subendocardial necrosis consistent with a reperfusion injury, but no significant coronary artery disease.

The second case involved a 54-year-old male subject (BMI 26.5) who, after using PCP and in an intoxicated state, sexually assaulted an elderly woman in front of her son. The son wrestled the subject down with the subject on top of him in a chest-to-chest position, where he held the subject prone with his arms over his neck and shoulders until he stopped struggling and the police arrived. Upon arrival, the police noted that the subject was unresponsive and without a pulse. The police began Cardiopulmonary Resuscitation (CPR) until EMS arrived.

Upon arrival, the EMS noted hyperthermia and continued the CPR using a Lucas chest compression device and began ACLS measures. The subject continued with PEA and asystole until a pulse was regained. He was transported to the hospital. **Electrocardiogram** (EKG) showed a T-wave inversion and a right bundle branch block; laboratory testing showed increasing Troponin levels. He remained comatose, and was declared brain dead a week later. Autopsy revealed minor injuries consistent with the struggle. Cardiovascular consultation revealed a 360gm heart with a focal reperfusion necrosis, moderate dysplasia of the AV nodal artery, and mild coronary atherosclerosis. Neuropathology consultation revealed acute hypoxic injury with tonsillar herniation.

Both cases involved subjects using PCP, who were restrained in a prone position, which would result in decreased ventilation and an inability to blow off Carbon Dioxide (CO<sub>2</sub>). There was also a decrease in cardiac output by increasing intrathoracic pressure and decreasing venous return. This study believes this led to arrhythmias of PEA and asystole and death. Despite the assertions of Vilke et al., these cases appear to confirm the dangerousness of prone restraint and support the notion of prone restraint cardiac arrest.

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### Prone Restraint Cardiac Arrest, In-Custody Deaths, Police-Involved Deaths

## H131 The Role of Mast Cells in Deaths Associated With Fatal Asphyxia: An Immunohistochemical Study Utilizing the CD117 Marker

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**Learning Overview:** Attendees will get a better knowledge of the essential role of Mast Cells (MCs) in acute hypoxia. Asphyxia patients had significantly more CD117+ MCs than controls, especially near arteries in the peri-alveolar and peri-bronchial regions. This may assist in identifying instances of acute asphyxial death that may go undetected otherwise.

**Impact Statement:** This presentation will educate attendees and members of the forensic science community about MCs' usefulness in acute hypoxia. This research discovered that asphyxia victims had significantly more CD117+ mast cells than control subjects, especially in the peri-alveolar and peri-bronchial regions next to arteries. Asphyxia results in a rapid and extensive enrollment of peri-bronchial and peri-alveolar mast cells in the lungs, which is facilitated by explicit chemotactic soluble macrophage-derived substances. CD117+ MCs indicate a twofold increase in peri-bronchial MCs in asphyxial deaths. Thus, these results establish the essential role of MCs in acute hypoxia, a finding that merits further study since it may assist in the detection of instances of acute asphyxial death that would otherwise go undetected.

Asphyxia-related deaths have always been a challenging task in the specialty of forensic pathology. Asphyxia-related deaths account for a significant fraction of the cases handled by forensic pathologists.

Asphyxia is the second-leading cause of mortality after mechanical damage.<sup>1</sup> Cases of death resulting from pressure effects on the neck and chest where soft tissue injuries are limited or non-existent can lead professionals to an erroneous conclusion; apart from helpful macroscopical signs (e.g., strangulation mark, cyanosis, petechial hemorrhage, and lung edema), recent literature indicates that prolonged asphyxia is sufficient to induce an increase in MCs.<sup>2</sup> Inflammatory cells migrate from the bone marrow to the lungs, aiding in the diagnosis of fatal asphyxia death. HIF-1 $\alpha$ , a key regulator protein, is released from lung tissue capillaries during catastrophic hypoxia circumstances, as previously demonstrated in Immunohistochemistry (IHC) research.<sup>3</sup>

The present study analyzed lung samples from 164 medicolegal autopsy cases, including 57 asphyxia/hypoxia deaths and 107 controls (non-asphyxia deaths). Peri-bronchial, peri-vascular, and peri-alveolar MCs were detected using CD117 antibody, and the average of MCs in each of these locations was noted in each case. The results indicated a statistically significant increase in peri-bronchial and peri-alveolar MCs in fatal asphyxia deaths, including those caused by hanging, drowning, or postural asphyxia. Peri-bronchial MCs in lung sections of asphyxial deaths were in the range of 0.2–5.4, and in non-asphyxial samples were in the range of 0.0–2.2. Peri-alveolar MCs in lung sections of asphyxial deaths were in the range of 0.0–0.6, and in non-asphyxial samples were in the range of 0.0–0.2. This data suggest that MCs play an important role in fatal hypoxia-related mortality, and CD117 may be a reliable marker for the detection of MCs in asphyxial deaths. It is worth mentioning that asphyxial fatalities induced by hanging, drowning, or postural asphyxia generate significant findings regarding the number of CD117+ MCs.<sup>4</sup> These findings were shown to be statistically significant. It could be very beneficial to forensic pathologists tasked with differentiating fatal asphyxia fatalities from other causes of death.

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### Asphyxia, Mast Cells, Immunohistochemistry

## H132 A Suicidal Transnasal Hunting Crossbow Bolt Head Shot: A Case Report

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**Learning Overview:** Head injuries that appeared to be specific to a crossbow bolt were identified using Postmortem Computed Tomography (PMCT).

**Impact Statement:** The relevant injuries in this case benefited greatly from PMCT and the way it did will be discussed in this presentation.

**Case:** A 31-year-old man was found dead inside his firmly locked flat on the second floor after he had been missing for several weeks. His body appeared to lie on the floor, with a hunting bolt stuck in a wooden cupboard door above him as if shot with him sitting down.

Next to him, a crossbow with a longish wooden branch close to the crossbow trigger, indicating an ostensibly used trigger mechanism. Upon first inspection, the head appeared to exhibit a single penetrating injury to the left occipital region. Clothing and the remainder of the skin of the body appeared without perforating defect or injury. Consistent with a considerable degree of postmortem decomposition, the body lacked most facial and frontal scalp skin, whereas these soft tissues appeared severely degraded but only partly revealing the bone underneath.

The body was admitted to the Institute of Forensic Medicine for identification and for injury clarification using PMCT. The extended inspection of the premises revealed several handwritten pages that were interpreted as a suicide note and even contained the indication that a crossbow shot through a facial orifice may be attempted. The crossbow identified was a hunting crossbow. The bolt that was retrieved contained a round bolt shaft of around 45cm in length and 9mm in diameter. The single arrow tip was about 2mm thick, and its triangular shape was up to 26mm wide at its widest location. The tip mount appeared damaged, and soft metal wire appeared to have come partly undone. The partly fractured hard plastic straight fletching structures were arranged at symmetric 120-degree angles around the shaft circumference and protruded from the shaft surface by up to 12mm, with no apparent index/cock. PMCT showed slit-like comminuted defect fractures of the left sphenoid bone, matching the bolt arrow in extent and vertical orientation, as well as matching the fletching. There was a penetrating skull fracture with outward beveling and attached fracture fragments that were outwardly angled. The defect measured about 33.6 x 13.1mm; it contained additional outward pelleting laterally of 4–6mm each, matching the dimensions of the bolt fletching. The overall fracture constellation was different from a typical firearm injury in that the bone defects of the left sphenoid and left occipital bone matched a vertically oriented arrow, and there were subtle fractures matching the fletching of the arrow even at what was determined as the skull exit injury.

**Discussion:** Fatal crossbow injuries are rare. While literature suggests that crossbow shots and firearm injuries may appear similar at least to a degree, this case provided differentiating features in fracture appearances. The overall setting of this case was that of a suicide, and the overall setting did not allow for many other serious hypotheses to be supported. The literature documents many cases with bolts *in situ*. Now seeing as how crossbows can be purchased without a license and given their serious injury potential, not only suicides but accidents and homicides may have to be expected. In cases of bolts placed adjacent to a body or in case of missing bolts, the burden on investigation and evidence may be considerably higher, so learning from what appears to be situationally “validated” cases such as this seems legitimate and relevant. In this case, PMCT appeared to be of great value.

### Virtopsy, Postmortem Computed Tomography, Crossbow

## H133 Bad Manners: Suicide by Starvation

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**Learning Overview:** After attending this presentation, attendees will understand the mechanism and manifestations of death due to starvation and appropriately address the challenges of determining manner in such cases.

**Impact Statement:** This presentation encourages attendees to consider the pathologic presentation of starvation as a mechanism in an unnatural death.

Medical examiner offices provide a unique insight into epidemiologically significant local trends, emerging social and environmental threats, and the psychosocial health of the community.<sup>1</sup> This study presents an unusual case of suicide by starvation in an otherwise healthy 51-year-old male.

Scene investigation of the decedent's residence revealed a well-stocked pantry and refrigerator. There were no readily apparent signs of illicit drug use or prescription medications. The decedent's laptop had a browser open with "fumes in bag suicide" typed in the search engine, though no gas tank was found in the residence and there was no bag around the head or deposited near the decedent's body. Numerous handwritten notes were present with expressions of suicidality and suggested attempts at water deprivation. Surviving family members endorsed concerns for the decedent's current suicidal ideations, citing the previous suicide of his twin sibling and numerous recent social and economic stressors, as well as confirming that the decedent's temperament aligned with this protracted means of suicide by starvation—his mother characterized his personality as determined and stoic. The autopsy findings reflected the known manifestations of starvation: a markedly low Body Mass Index (BMI) of 12.7kg/m<sup>2</sup> and organ weights approximately 20–50% less than expected with preservation of brain weight at 1,490 g.<sup>2</sup> No mass lesions, neoplastic processes, or systemic inflammatory diseases were identified by gross or microscopic examination. A basic forensic toxicological panel performed on postmortem femoral blood was negative for ethanol, common drugs of abuse, and select therapeutic medications. Vitreous fluid analysis demonstrated a pattern consistent with hypertonic dehydration, with a sodium level of 160mmol/L, chloride of 120mmol/L, potassium of >20mmol/L, creatinine of 4.2mg/dL, and urea nitrogen of >100mg/dL.<sup>3,4</sup>

The scene investigation, family history, and social factors heavily contributed to the forensic interpretation in this case, along with negative toxicological results. Caloric insufficiency in adults can arise due to natural causes (e.g., cachexia in the setting of chronic disease) or non-natural causes, as seen in cases of psychiatric disease, elder neglect, torture, or politically motivated food refusal (i.e., hunger strikes), and the attribution of manner of death reflects this diversity.<sup>1,5</sup> Recognition of the antemortem behaviors, autopsy findings, and social circumstances surrounding death by starvation is crucial in attaining a holistic and accurate cause and manner of death.

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### Starvation, Suicide, Manner of Death

## H134 Suicide and Mental Health: Observations From a Coroner/Medical Examiner Office

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the purpose of death certificates and the lack of their contribution to vital mental health statistics.

**Impact Statement:** This presentation will impact the forensic science community by assessing the lack of mental illness documentation on death certificates in cases of suicide at a coroner/medical examiner office.

Overall, the medical community (and likely the community at large) considers a diagnosis of mental illness as a condition that carries a significant risk of suicide ideation, attempt, and completion.<sup>1</sup> However, vital health statistics are often derived from death certificates that rarely include a mental health diagnosis. Researchers would benefit from obtaining data from the primary source, such as the coroner and/or medical examiner's office that investigates the majority of these deaths, but the current medicolegal death investigation system does not allow for this to occur easily. Standardization of death certification practices overall would improve the quality of mortality data; this is especially true in cases of suicide where assessing the true impact of mental illness would benefit clinicians, patients, and families.

Suicide is a manner of death that is captured as a structured field on the death certificate as one of only five options. Causes of death, listed in Part I of the death certificate, capture events that directly resulted in the death. Part II of the death certificate is used to collect information on contributing factors to death; although it is very uncommon for mental health diagnoses to be listed. One study concluded that only 5.5% (124) of death certificates had any information in Part II.<sup>2</sup> The aim of this project was to utilize a primary data source, like a coroner/medical examiner office's database, to investigate those individuals who died of suicidal means and present observatory details regarding the prevalence of mental illness (namely Post-Traumatic Stress Disorder (PTSD), depression, bipolar disorder, and others) reported in these deaths.

The database at the Jefferson County Coroner/Medical Examiner's Office (JCCMEO) in Birmingham, AL, was queried for decedents with a manner of death of suicide between January 2013 and May 2021. Demographic information, circumstances surrounding death, and reported history of mental illness were collected from case information within the database. Information from the scene, including witness accounts and interviews with family and friends at the time of the death, were obtained from narratives written by medicolegal death investigators.

A total of 757 deaths certified with a manner of death as suicide were identified. The overwhelming majority were due to the use of a gun (68%,  $n=513$ ), followed by hanging (18%,  $n=137$ ). A small percentage (2%,  $n=14$ ) of the decedents had a reported history of PTSD, 43% ( $n=325$ ) had a reported history of depression, and 6% ( $n=44$ ) of bipolar disorder. In total, 50.6% of the decedents had a reported history of some sort of mental illness. However, none of this information was captured on the death certificate, which highlights the severely compromised ability to accurately study the association of mental illness and suicide.

Future studies will include comparing witness and family reports of mental illness with available medical records. Additionally, a strategy will be developed for future coroners/medical examiners to consider listing a mental health diagnosis in Part II of the death certificate. This information will also help better inform our clinical colleagues of the association of mental illness and suicide when treating their patients.

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### Suicide, Mental Illness, Death Certification

### **H135 Web-Enabled Suicide Using Sodium Nitrite: An Old Forensic Challenge in New Clothes**

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**Learning Overview:** This presentation will recontextualize intentional sodium nitrite poisoning and provide attendees with the tools to increase awareness of clinicians and forensic colleagues to the challenges presented by methemoglobinemia in patients and in the postmortem period.

**Impact Statement:** This presentation will provide new impetus to collect further, good-quality evidence regarding sodium nitrite and methemoglobinemia in the postmortem period in order to build a body of knowledge that will aid in future forensic analyses.

Suicide by sodium nitrite ingestion has been long described as a rare fatality.<sup>1</sup> However, the portrait of the last couple of years appears to be different due to a worldwide increase of cases.<sup>2-11</sup>

This presentation describes the case of an 18-year-old man who intentionally ingested an unknown quantity of sodium nitrite together with antiemetic medication. In the same period, two other suspected cases of self-poisoning with sodium nitrite occurred in Italy. As previously seen in the literature, instructions for the suicidal procedure were found on the Internet<sup>7</sup>.

Autopsy findings revealed an asphyxial pathophysiological mechanism of death through cyanotic changes of the nails, as well as internal signs of asphyxia such as subepicardial and subpleural petechiae, pulmonary emphysema, and congestion of the lungs. Toxicological analysis showed a concentration of summed nitrite and nitrates of 1,400 µmol/L in the blood sample collected immediately after death and a concentration of methemoglobin of 27.7% in the blood sample collected during autopsy, performed 36 hours after death.

Both sodium nitrite and methemoglobin analysis pose a challenge for forensic sciences. The rapid oxidation of the salt and the particular characteristic of the blood in the postmortem period can lead to false negatives.<sup>5,12</sup> After death, methemoglobin can both decrease due to the action of enzyme MetHb-reductase or bacterial contamination, and increase because of decomposition of the body or auto-oxidizing phenomena.<sup>3,13,14</sup> Blood sample storage conditions such as temperature, quality of blood, preservative, and time between sampling and the analysis can all influence the validity of the measurement<sup>5,13,14</sup>

In this case, autopsy was performed 36 hours after death; the actual storage conditions of the body were not known. Blood was conserved in sodium fluoride and potassium oxalate and was left at room temperature for six hours after collection before freezing. As already described in previous works, the percentage of methemoglobin in this blood sample was lower than the values indicated as fatal in the existing literature.<sup>3,5</sup> With current knowledge, the absolute amount of methemoglobin could not be used to determine the cause of death by itself; it is necessary to confirm the diagnosis through the concentration of sodium nitrite and nitrate in the blood. Much more effort should be addressed in this topic to best inform both clinicians and forensic specialists.

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#### **Sodium Nitrite, Suicide, Methemoglobinem**

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## **II Schema Mode Work, Limited Reparenting, and Empathic Confrontation in Solution-Focused Therapy (SFT) in the Treatment of Forensic Late Adolescent Patients With Antisocial Personality Disorders Who Committed Offenses and in Precautionary Measures in the Community**

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**Learning Overview:** The goal of this presentation is to demonstrate how the Schema Therapy Model can represent a very effective approach to treat forensic late adolescent patients with antisocial personality disorders or significant pathological cores of personality.

**Impact Statement:** With this type of psychotherapeutic approach, it is also possible to treat patients and the forensic population who have been, until recently, considered very difficult and sometimes impossible to treat with more traditional psychotherapeutic techniques of first and second generation. This presentation will impact the forensic science community by helping attendees be able to communicate the strong points of the Schema Therapy Model. They will bypass the limits of Standard Cognitive Behavioral Therapy in the treatment of late adolescent forensic patients affected by antisocial personality disorder or other types of personality disorders who committed various types of offenses and crimes.

**Introduction:** This presentation takes as its thesis the confirmed effectiveness of the Schema Therapy Model for the forensic population with severe dysfunctional coping modes and late adolescent patients who have committed offenses. The Schema Therapy Model was adapted by D. Bernstein, A. Arntz et al., to meet the specific requirements of the forensic population. These patients are often affected by severe personality disorders and are characterized by significant fluctuating emotional states. SFT treats these states with the aim of integrating them through limited reparenting and empathic confrontation techniques. Recent research suggests that standard cognitive and behavioral approaches are only of limited effectiveness in forensic patients with personality disorders. SFT may represent a very effective psychotherapeutic approach for late adolescent forensic patients with antisocial personality disorders.

**Results:** Schema Mode Work, limited reparenting, and empathic confrontation, used as the core of SFT practice in the treatment of more severe personality disorders, confirm their effectiveness in personality disorders in forensic settings and late adolescence. The Schema Mode model was expanded to include new dysfunctional coping modes, bully and attack, predator, paranoid over-controller, self-aggrandizer, and conning and manipulative personality traits that appear to be particularly frequent in late adolescent and adult forensic patients with antisocial personality disorders.

Schema Modes often play themselves out in a predictable pattern. In some instances, these temporal sequences of unfolding dysfunctional coping modes may help to explain the events leading up to and culminating in the committing of crimes and offenses (robbery, mafia crimes, assaults, drug dealing, attempted murder, theft, illegal carrying of arms, etc.). SFT principles and methods have been integrated into each phase of treatment from the start to the completion. The goal of forensic ST is to reduce the patient's reliance on maladaptive coping modes by breaking through the patient's emotional detachment to access and heal their "vulnerable child mode".

During psychotherapy, the empathic confrontation with patients' dysfunctional coping modes, such as paranoid over-controller, bully and attack, and conning and manipulative modes, and limited reparenting with imagery rescripting technique used in past-present emotional bridge exercises, showed their effectiveness in the treatment of patients and in strengthening their functional ways. This allowed the patients to re-enter society in a shorter time and to make good use of therapy by modeling themselves on increasingly adequate, balanced, and healthy behaviors.

At the end of the psychotherapy, the frequency of activation of early maladaptive schemas was diminished, much as the frequency of activation of dysfunctional coping modes. These results were also demonstrated by the scores obtained by the Young Schema Questionnaire and the Short Mode Inventory administered in the post-treatment phase.

**Conclusion:** SFT for adolescent forensic patients and in particular for patients diagnosed with antisocial personality disorder confirm how this psychotherapy approach, its core techniques, and the importance attributed to the therapeutic relationship is very effective for this type of patient. Mostly, the mode work with bully and attack and paranoid over-controller modes allowed the patients to achieve improvements in their lives and in the forensic context. The ongoing research into this model will provide more insight into the mechanism that explains the apparent effectiveness of SFT in forensic personality disorder patients, including some psychopathic patients.

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### **Adolescent, Antisocial Personality Disorder, Schema Therapy**



## I2 Housing Conservatorship: New Paradigm or Unfunded Boondoggle?

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**Learning Overview:** The goals of this presentation are to: (1) summarize California Welfare and Institutions Code (WIC) §5450, which establishes a “Housing Conservatorship;” (2) review the primary purposes of this legislation; (3) discuss the evidence and opinions for and against the “Housing Conservatorship” statute; and (4) examine the current state of implementation of this pilot program.

**Impact Statement:** This presentation will analyze California WIC §5450, a recent law that establishes a new kind of conservatorship targeting individuals with serious mental illnesses and substance use disorders, who have had eight or more involuntary psychiatric detentions in a 12-month period. This presentation will review the implementation of the “Housing Conservatorship” pilot program and its role in treating individuals with serious mental illness and substance use disorders.

In 2019, 151,000 Californians were homeless, 23% of whom were severely mentally ill, and 17% of whom had a chronic substance abuse disorder.<sup>1</sup> Officials across the state have struggled to meet the needs of homeless individuals with untreated mental illness and substance use disorders who decline services for housing or treatment. California civil commitment laws under the Lanterman-Petris-Short (LPS) act allow for individuals with a mental illness who are “gravely disabled” (unable to provide food, clothing, or shelter for themselves) to be placed under a conservatorship.<sup>2,3</sup> The conservator is empowered to decide treatment and housing for the conserved individual (conservatee).<sup>3</sup>

Officials in San Francisco, CA, perceived two flaws with LPS conservatorship: (1) although it allows chronic alcohol use as a criterion for determining grave disability, other substance use is not considered; and (2) it requires a series of involuntary psychiatric holds in the hospital prior to the individual being conserved.<sup>4</sup> The ineligibility of other substance use as a criterion for determining grave disability was problematic given that methamphetamine-related diagnoses exceeded alcohol-related diagnoses by a greater than 2:1 ratio among individuals presenting to psychiatric emergency services in San Francisco.<sup>4</sup> Individuals with substance use disorders were frequently detained for psychiatric treatment. However, after receiving a short period of involuntary treatment, they no longer met grave disability criteria under LPS and thus could not be conserved. They would decline voluntary treatment, only to re-present involuntarily to psychiatric emergency services when they were gravely disabled in a state of intoxication and psychiatric decompensation.<sup>5</sup>

Scott Weiner, State Senator from San Francisco, introduced and passed Senate Bill 1045 (SB-1045) in 2018, and Senate Bill 40 (SB-40) in 2019. These established a pilot program authorizing the counties of San Francisco, Los Angeles, and San Diego to implement a “Housing Conservatorship.”<sup>6,7</sup> Supporters hoped this form of conservatorship would end the revolving door of involuntary psychiatric detention, psychiatric stabilization, refusal of services, continued substance use, and psychiatric decompensation.

Opponents argued that California WIC §5450 expanded the number of conservatees without providing additional funding for supportive housing, mental health services, or substance use treatment.<sup>4,5</sup> Homeless and disability rights advocates voiced concerns about infringements on civil liberties. They asserted that Assisted Outpatient Treatment (AOT), a form of court-ordered outpatient treatment, could encourage individuals to engage with treatment while respecting patient autonomy.<sup>4,5</sup>

To date, only San Francisco has started implementing “Housing Conservatorships.”<sup>8</sup> San Diego and Los Angeles Counties withdrew from the pilot, citing difficulties such as the need for a centralized database to track the number of times a particular individual has been placed on a psychiatric hold.<sup>9,10</sup> As San Francisco implements this recently enacted conservatorship, it remains to be seen whether it emerges as a new paradigm for treating severe mental illness and substance use in the chronically unhoused population or ends as an unfunded boondoggle.

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### Conservatorship, Homelessness, Substance Use

### I3 From Neomycin-Bacitracin-Polymyxin to Sexually Transmitted Diseases (STDs): The Significance of Interpretation and Attentiveness When Treating Developmentally Disabled Patients With Severe Mental Illness

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**Learning Overview:** This report seeks to highlight the importance of attentiveness among health care workers to be able to interpret the sometimes confusing requests of patients.

**Impact Statement:** Patients with concomitant severe mental illness and intellectual disability struggle to get adequate medical care given their inability to advocate for themselves as a result of their cognitive disorganization and limited vocabulary. This presentation will seek to expand the forensic community's awareness of the importance of recognizing subtle cues from intellectually disabled individuals that may identify indicators for abuse and trafficking.

Health care disparities for people with mental illnesses, due to a variety of factors including access and quality of care provided, result in poor health outcomes and often premature death.<sup>1</sup> Evidence also suggests that many patients suffer from a dual diagnosis of psychiatric disorders, such as schizophrenia and intellectual disabilities.<sup>2</sup> Such individuals are all the more vulnerable and often do not receive the help they need due to their mental health and behavioral problems.<sup>3</sup> What's more, systemic reviews of patients who have suffered from human trafficking suggest that many suffer from mental illnesses and have longer psychiatric admissions.<sup>4</sup>

Ms. A, a 20-year-old homeless female with unknown psychiatric history was brought to a psychiatric emergency department by police after being found in a driveway mumbling to herself incoherently. Ms. A was eventually transferred to an inpatient psychiatric ward for further stabilization with psychotropic medications. Over time, she began using words to communicate, experienced less auditory hallucinations, and was less frequently seen responding to internal stimuli. Around one month post-admission, as the patient was pre-rounded on by medical students, she mumbled in a low voice "Can I get Neosporin®?" Upon further questioning, she indicated it was requested for use "down there," pointing to the genital region and mumbling "Someone cut me down there." When questioned by whom, when, and why she was cut, the patient remained selectively mute and then asked again for Neosporin®. Her knowledge of the word Neosporin® as well as her body language and decision not to disclose further information was concerning. This suspicion was what prompted the rounding medical student to look further into the request. Fortunately, the patient agreed to physical examination by the attending physician who observed vaginal and anal lesions. This led to a gynecology consult where Sexually Transmitted Disease (STD) testing was suggested, given observations and suspected risky sexual behavior with numerous partners, as this patient, suffering from schizophrenia, tried to fend for herself on the streets. Often, such patients are victims of human trafficking. Upon testing, the patient's HIV and Hepatitis panel were non-reactive, but she was diagnosed with chlamydia, gonorrhea, HPV anogenital warts, and syphilis. An outpatient cryotherapy appointment was made to address the warts, while the other diagnoses were treated onsite. The infectious disease team that was consulted indicated that a lumbar puncture was necessary, which later resulted negative for neurosyphilis. Ms. A was also scheduled for outpatient labs for repeat HIV and Rapid Plasma Reagin (RPR) in one and three months respectively. During this process, the Department of Public Health was consulted regarding the patient's previous STD diagnoses and treatments and was later notified of her current reportable diagnoses.

In conclusion, from a simple request for Neosporin, this patient was consulted by two different medical teams, diagnosed with four sexually transmitted diseases, treated for three of them inpatient, and arranged for three outpatient appointments for both treatment and further diagnostic testing. Her status as a possible victim of human trafficking was also reported to the appropriate authorities.

Thus, careful attention must be made to terms and requests made by psychiatric patients, particularly when they are in reference to medications or symptoms seemingly unrelated to their psychiatric diagnosis. By doing so, health care providers can provide holistic care by addressing medical concerns that may have in fact stemmed from the patient's lack of opportunities given their mental illness. Increased awareness can also help identify indicators for abuse and trafficking that can then be used to help the victims.

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#### Severe Mental Illness, Neurodevelopmental Disability, Attentiveness

## I4 A Case of Neonaticide With Homicidal Defenestration of the Child

Bernat-Noël Tiffon Nonis, PsyD\*, Universitat Abad Oliba, Barcelona, Spain

**Learning Overview:** The novice assistant can learn, retain, and implement psychological evaluation techniques in juvenile homicide cases. The experienced assistant can implement new or renewed insights into the methodology of psychological evaluation techniques in cases of homicides perpetrated by minors.

**Impact Statement:** The impact that can influence the forensic scientific community is to illustrate the psychology and/or psychopathology of a singular case of homicide perpetrated by a new mother with her baby only a few minutes old.

**Introduction:** When a parent kills their own child, it is known as filicide. Such a criminological situation always originates a great social, legal, and media impact.<sup>1-4</sup> Calzada et al. consider “as neonaticide the death of a child in the first 24 hours.”<sup>5</sup>

**Case:** The proven facts in the case of a 19-year-old woman were, as drafted and stated in the body of the sentence, as follows: “. . . at an unspecified time, but between the afternoon and the evening, she gave birth in the bathroom of her home to a female baby that came to breathe autonomously; and immediately after the birth, she threw it out of the bathroom window that overlooks an inner courtyard of the property from a height of 74.97 feet. As a result, the newborn suffered severe polytrauma with the destruction of vital brain centers, which caused her death.”

**Discussion:** From the legal point of view and as stated in the sentence, the jury considered as proven that the baby was thrown and defenestrated by the defendant from the window. From the psychological point of view, and as a result of the psychometric examination carried out, the personality profile of the victim is compatible with a personality characterized by an acute and singular fragility, weak and sensitive psychoemotional instability, avoidant-dependent-melancholic, which weakens in situations interpreted subjectively and paranoically as critical and/or acute.

**Conclusion:** This study describes a singular case of neonaticide of a young mother with a dysfunctional personality and suffering from an acute psychoemotional instability. It is difficult to describe psychologically what was the mother’s will to perpetrate the acts since only the victim knows what her most intrinsic motives were.

As it is clear from the sentence, all the experts who testified regarding the psychological state of the victim agreed that the victim did not present any type of serious mental disorder for which she could benefit from an exonerating circumstance or an attenuation of the criminological penalty. Thus, and following the ruling of the sentence, this meant the consideration of being the responsible author of the crime of murder with the aggravating circumstance of kinship to the penalty of 20 years of imprisonment. Likewise, in order to develop preventive strategies in cases of neonaticides and filicides, it is notoriously difficult to establish and apply due to the heterogeneous dynamics between the perpetrator and the victim.<sup>6</sup>

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### Neonaticide, Filicide, Personality Disorder

## I5 A Homicidal Outburst and Preservation of the Corpse at Home

*Bernat-Noël Tiffon Nonis, PsyD\*, Universitat Abad Oliba, Barcelona, Spain*

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**Learning Overview:** After attending this presentation, the novice assistant will have learned, retained, and be able to implement psychological evaluation techniques in homicide cases. The experienced assistant will be able to implement new or renewed insights into the methodology of psychological evaluation techniques in cases of homicides.

**Impact Statement:** The impact that can influence the forensic scientific community is to illustrate the unique psychology and/or psychopathology of a perpetrator who, after murdering his ex-partner, suffered from Borderline Personality Disorder (BPD), and kept his corpse buried for some ten months in his own home.

**Case:** The patient was a 55-year-old single man with no children and no known psychopathological clinical history who was in a companion relationship with a woman who had been his previous affective-emotional partner in the past. Due to their financial situation and the woman's needs, who was affected by BPD, he decided to take her into his home in order to help her with her basic needs for daily life. Their relationship was characterized by continuous interpersonal conflicts, the result of the psychic dysfunction of BPD suffered by the woman.

In one of these frequent discussions that used to periodically characterize the dynamics of the couple, and as a result of a sharp and heated argument in which the reported was subjected to strong psycho-emotional pressure with the presence of frequent insults, humiliation, and psychological abuse (and even physically slapping him, pulling his hair, and causing scratches) for which he felt subjected by her, he decided to kill her in a psychological state of rapture.

According to the words of the reported (now victimizer), “. . . *the situation was impossible and unsustainable*” so that “*in the heat of the argument, he does not remember if she tried to cover herself or if she did anything to defend herself from the ax that he took and with which he hit her two or three times.*” He added that “*he does not know if she died on the spot, but he does know that he left the victim's body in the shower tray for two or three hours.*”

After committing the crime, he proceeded to bury the victim's body in his own home and, after 24 hours, he reported her disappearance to the police. The assailant kept the victim's body buried in his home over a ten-month time frame until it was finally discovered through the police geolocator.

**Discussion/Conclusion:** The description of the case illustrates the situations in which a subject affected by BPD can originate in the usual dynamics of his interpersonal relationships characterized by the presence of continuous conflicts and arguments.

On this occasion, the (double) victim of BPD and crime was a subject who, due to the continuous and periodic level of conflict, was seized by negative emotions and the frustration of the moment. The low threshold of patience made him break his inhibitory mechanisms of behavior to materialize in a harmful psycho-emotional outburst with a fatal outcome.

The experienced assistant can implement new or renewed insights into the methodology of psychological evaluation techniques in cases of homicides perpetrated by victims of BPD.

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### Burial of Corpse at Home, Borderline Personality Disorder, Outburst

## I6 Death Due to Autoerotic Practices: Evidence for Diagnosis Resulting From a Systematic Review

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of autoerotic death phenomenon.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence resulting from a systematic review on the topic and useful to establishing the diagnosis of autoerotic death.

Autoerotic death is defined as “accidental death that occurs during individual, usually solitary, sexual activity in which some type of apparatus that was used to enhance the sexual stimulation of the deceased caused unintended death.”<sup>1</sup> An important point in cases of autoerotic death is that the manner of death is accidental.

In this context, autoerotic asphyxia death refers to “fatal episodes that result from asphyxia, thus maintaining the distinction from rarer cases involving quite different mechanisms” and represents the predominant manner and mechanism of the reported cases of autoerotic death.<sup>1</sup> Specifically, autoerotic asphyxia is defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)* as a particularly dangerous form of masochism, which is considered a paraphilic disorder, associated with transvestism in a substantial proportion of fatal cases, which is at risk of accidental death while practicing.<sup>2</sup> Historically, it is documented that prostitutes have been experts in sexual asphyxiation for a long time. During the Victorian era in London, men could satisfy their sexual urges through controlled hangings in the “Hanged Men’s Club.”<sup>3</sup> The origin of this practice was traced to the Celts, but early accounts have also been found in various ethnic groups, including the Eskimos, Southeast Asians, and Yaghans of South America.<sup>4,5</sup>

Other less common autoerotic deaths, sometimes referred to as “atypical” because asphyxiation is not a feature, include deaths due to electrocution, hemorrhagic shock, and fatal air embolism.<sup>6-9</sup> The diagnosis of autoerotic death is not always so simple, especially in the absence of typical death scene characteristics.

The goal of this presentation is to describe forensic features involved in autoerotic death cases, focusing on life and environment of the victim, the circumstances of death, and autopsy findings that may be effective in the determination of the manner of death in equivocal cases and to highlight the most common causes of death. Finally, this review proposes a practical medicolegal approach to support the forensic professionals in making diagnosis in the hypothesis of such cases and, therefore, to distinguish accidental, suicidal, and homicidal death.

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### Paraphilic Disorders, Autoerotic Deaths, Crime Scene Investigation (CSI)

### I7 A Five-Year Review (2016–2020) of Intimate Partner Homicides at the Harris County Institute of Forensic Sciences

Jennifer L. Ross, MD\*, Harris County Institute of Forensic Sciences, Houston, TX

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the incidence and demographics of Intimate Partner Homicides (IPH) over a five-year period (2016–2020) at the Harris County Institute of Forensic Sciences in Houston, TX.

**Impact Statement:** This presentation will impact the forensic science community by comparing, over a five-year period within Harris County, TX, the number of IPH, the demographics of both the victim and perpetrator, the type of weapon(s) used, and the incidence of suicide and/or injury/death of others at the time of the homicide.

IPH make up a subset of deaths due to domestic violence. Domestic violence homicides can be subdivided into non-IPH (parent, child, sibling, other family member, third party to current/former intimate partner, and roommate) and IPH (current/former spouse, current/former intimate partner). A recent study showed rates of IPH are increasing.<sup>1</sup> It is well known that males are far more likely to be both perpetrators and victims of homicide and that women have a higher rate of victimization than offending. Black women are two-and-a-half times more likely to be killed by an intimate partner than White women.<sup>2</sup> A previous study found that both sexes employ firearms most often with nearly three-quarters of male offenders and nearly half of females using a firearm.<sup>1</sup>

The incidence of IPH, victim and offender demographics, victim-offender relationship, and weapon used were reviewed from deaths due to domestic violence over a five-year period (2016–2020) at the Harris County Institute of Forensic Sciences. The percentage of IPH cases out of all homicide cases has increased slightly from three percent in 2016, four percent in 2017, three percent in 2018, five percent in 2019, and five percent in 2020. The cases have similar results as other studies, with females more commonly victims, Blacks more common as both victims and perpetrators, and firearms as the most common weapon used. The results are important for the local community as they can lead to improved prevention efforts.

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#### Domestic Violence, Intimate Partner Homicide, Harris County

## 18 Homicides Committed by Delusional Patients in the Early 20<sup>th</sup> and 21<sup>st</sup> Centuries: A Study Conducted in a French Secure Unit

Eloise Provoost, MD\*, Paris Center Psychiatry Unit, Paris, France

**Learning Overview:** The aim of this presentation is to provide an analysis of psychotic homicide and identify the clinical features of delusions that lead to that type of homicide. Attendees will have a better understanding of the characteristics of homicides committed by delusional patients.

**Impact Statement:** This work completes available data by describing two groups from different centuries and exploring delusional themes. This presentation will impact the forensic sciences by improving the understanding of psychopathology of psychotic homicide.

Homicides committed by delusional patients are the object of a rich scientific literature, which puts the risk of such acts occurring into perspective when analyzing the offenders' sociodemographic and clinical characteristics.<sup>1-5</sup> However, few articles detail the themes and mechanisms underlying those patients' delusions.

To help bridge that gap, a retrospective descriptive study was conducted in a French secure unit, including two samples of delusional homicidal patients; one from near present day (2015–2019) and one from nearly a century ago (1910–1914). This study considered similarities observed in the literature (such as patients' sociodemographic profile, clinical data, and acting-out dynamics), but also explored the characteristics of delusion (such as theme, mechanisms, conviction to the delusion, and societal influence).

In the 2015–2019 sample, the typical patient profile was: single male (31.5 years old on average), without child, unemployed, and with psychiatric history (56.6%). A comorbidity of substance-use disorder was predominantly present (83.3%). Most patients suffered from schizophrenic disease (83%) with non-systematized delusions exhibiting multiple themes in 80% of cases. Four principal types of delusion were observed: persecutive (100%), mystical (43.3%), megalomaniac (30%), and bodily (30%). The mechanisms were interpretative, hallucinatory, and intuitive. There was a societal influence in 23.3% of the cases (most often terrorist acts). The acting-out characteristics were: no premeditation (70%), homicides committed in the victim's home, overkill (40%), knife use (80%), and no staging (in 86.6%). The victims were extra-familial in 62% of cases and intra-familial in 32% of cases (more often the parents).

The 1910–1914 historical sample revealed several differences compared to the near present-day sample: patients were older, more often married, and employed. There were more diagnoses of chronic delusional disorder (30%). Persecutory delusion was constant (100%) and the other delusional themes were the "intimate relationship" type (50%), jealousy, erotomaniac, and the bodily type (40%). The crime was more often committed with firearms (60%). Strangers were rarely victimized (9%); among intra-family victims (36.4%), uxoricides and fratricides were the only ones documented.

Considering the homicides from a preventive point of view, clinicians will be able to identify the key features of risk displayed by some of them. However, additional studies are useful to reinforce these findings and to further investigate the possibilities of prevention.

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### Delusions, Psychotic Homicide, Forensic Psychiatry

## 19 Hair Toxicology Identifies Greater Substance Use Than Self-Reported in High-Risk Youth Enrolled in the Adolescent Brain Cognitive Development<sup>SM</sup> (ABCD) Study

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**Learning Overview:** After attending this presentation, attendees will have learned how pre-adolescent and adolescent youth hair toxicology results relate to self-reported substance use and acute toxicology results.

**Impact Statement:** The results of this study indicate underreporting of substance use in youth at risk for substance use participating in a healthy development study.

**Aim:** A primary aim of the longitudinal ABCD Study<sup>®</sup> is to document substance use onset, patterns, and sequelae across adolescent development. As intentional and unintentional misreporting of substance use obscures meaningful relationships, objective measurement is needed. Hair toxicology tests provide objective historical substance use data, but are rarely investigated in youth. Here, objective hair toxicology results are compared with self-reported substance use in youth.

**Methods:** A literature-based substance use risk algorithm identified 696 ABCD Study<sup>®</sup> participants for hair sample collections between baseline and two-year follow-up (spanning ages 9–13 years) for laboratory analysis. Chi-square and *t*-tests assessed differences between participants' demographics, positive and negative hair tests, and self-reported drug use.

**Results:** Hair testing confirmed 121 of 696 (17%) hair samples positive for one or more substances (9.1% amphetamines, 5.3% cannabis, 1.9% cocaine, 3.6% cotinine, 1.4% alcohol, .3% opiates). Ninety-nine 9–13 year-old children were positive for one, 17 for two, 3 for three, and 2 were positive for four drug classes. Considering prescribed medications, 74 participants (11%) had positive hair tests incongruent with self-report. Participants with positive hair tests underestimated use via self-report ( $p < .001$ ), and participants with negative hair tests reported more sipping of alcohol than those with positive hair tests ( $p < .001$ ). Positive hair tests were more common in males ( $p = .009$ ), and those with lower parental education ( $p < .001$ ), lower income ( $p < .001$ ), and non-married parents ( $p < .001$ ). No participants with positive results self-reported recreational substance use consistent with toxicology results.

**Conclusions:** An alarming 11% of tested samples in 9 to 13 year-olds were positive for at least one unreported substance of abuse, suggesting underreporting in this population when participating in a national healthy development study. The scale of underreporting cannot yet be calculated given the selective way samples were prioritized for assay due to budgetary constraints. Expanded toxicology testing is key to characterize onset of substance use in studies of youth.

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### Children, Substance Use, Hair Toxicology



## I10 Death of a Doppelgänger: A Peculiar Case of Homicide

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**Learning Overview:** After attending this presentation, attendees will better understand the Doppelgänger phenomenon (or heautoscopy) in a case of atypical first-episode psychosis.

**Impact Statement:** This presentation will impact the forensic science community by showing that delusional mood (also known as *Wahnstimmung*), as a prodromal feature of an impending psychotic illness, can be dangerous if not properly recognized.

The Doppelgänger phenomenon involves the experience of a direct encounter with one's self, characterized by: (1) the perception of a figure having identical physical features with one's self; or (2) the apprehension that the perceived figure shares the same personality and identity. The Doppelgänger not only looks like the same person, it is his double. The perceptual element is usually a hallucination, though occasionally there is a false perception of an actual figure that may be involved. This phenomenon has been described in individuals with overwhelming fear, severe anxiety or intoxication, epilepsy, and in the transition between sleep and wakefulness.<sup>1</sup> It has also been reported in major psychoses. The fear of imminent death often precedes the Doppelgänger experience.<sup>2</sup>

This report presents a case of a 30-year-old man, Mr. XY, who was stabbed to death at the gym by Mr. XY, his "double". The aggressor and his victim, although not related, shared both name and surname, same age, professional activity, and place of work. Moreover, they attended the same sport center but barely knew each other.

A week before the homicide, the attacker, who had no history of psychiatric disturbance, had returned to live at home with his parents and sister, appearing more introverted and socially isolated in the aftermath of the separation from his girlfriend. One night he began to rant nonsense things and to be fixated on certain facts that happened on TV, mentioning international conspiracies. He revealed to his mother that he was afraid of being spied on by neighbors and that his family was in danger. Furthermore, he had perceived as a sort of "intuition" that her sister "had got pregnant, following a rape" perpetrated by the victim. His sister, while admitting having met him once through mutual friends, denied both a romantic relationship and the alleged pregnancy. The family consulted a general practitioner, who identified only a "depressive state" and prescribed benzodiazepines. The day before the agreed appointment with a psychiatrist, the murderer went to the gym and, as he crossed paths with the victim, killed him by stabbing him in the chest with a 25cm-long diving knife. The subsequent psychiatric evaluations in prison were suggestive of a psychotic condition.

This case is very unique in the scientific literature. In the most serious psychotic forms, the theme of the "double" calls into question not only the dissociative processes involved in the etiopathogenesis of the disorder, but also bio-psycho-social elements, as well as personal data that made the victim and the aggressor "identical."

In the context of such psychopathological functioning, the delusional mood (*Wahnstimmung*) that precedes the development of delirium is a sort of gateway to an impending psychotic illness, referring to delusional awareness or mood (atmosphere). In psychosis, splitting is the main theme and this influence was seen as an evil, foreign, apocalyptic, and unknown side that is no more recognized as belonging to itself also in the physical sense.<sup>3</sup> In such a situation, the only way to survive is the suppression of the one's own double as a defense against the disorganization of the self.

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### Psychosis, Doppelgänger, Homicide

## I11 Strangulation and Drowning: Homicide or Suicide?

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**Learning Overview:** The aim of this presentation is to focus on the crime scene investigation, autopsy findings, and histological studies to make the differential diagnosis between complex suicide and homicide.

**Impact Statement:** This presentation shows a rare type of complex suicide carried out by a combination of drowning and self-strangulation. The purpose of this case report is to focus the forensic community on the importance of a meticulous crime scene investigation and autopsy finding to establish the manner of death as suicide or homicide.

**Text:** The term “*complex suicide*” refers to a form of suicide in which more than one self-injurious action using different methods is applied.<sup>1</sup> A challenging task for forensic pathologists is being able to distinguish a complex suicide from a homicide. Therefore, circumstantial data, a meticulous crime scene investigation, a complete autopsy, and a histological study to search for signs of vitality of the lesions are fundamental.

**Case Study:** A 39-year-old woman was found dead floating in a water collection tank with a rope wrapped six times around her neck. An empty bottle of alprazolam and a kitchen knife were found near the collection tank. The external examination showed a ligature mark of the neck, a plume of froth at the mouth and nostrils, and eye and face petechiae. No other injuries were visible on the body. A Computer Tomography (CT) scan showed no traumatic injury. A complete autopsy was performed two days after her death. Cervical and thoracic organs were dissected with Gohn’s technique (en bloc). Froth and water were found in the tracheobronchial tree. No other significant findings were observed during the autopsy. Histological examination of all organs samples was performed using Hematoxylin-Eosin (H&E) staining. Lung samples showed acute emphysema, endoalveolar edema, and phytoplankton on phase-contrast microscope. Samples of vascular-nerve bundles of the neck and samples of sternocleidomastoid muscle showed no significant findings. Toxicological findings in blood samples included: alcohol 0.20g/l and alprazolam 138.6ng/ml. A plasma concentration range of alprazolam higher than 40ng/ml can be associated with central nervous system depressant effect.<sup>2</sup> After a few days, the police investigation found out that the victim suffered from depression and that she left a notebook in which she wrote her last wishes. Thanks to that complementary information, no signs of victim’s resistance on the body, and the results of the autopsy, the manner of death was established as primary complex suicide.

### Reference(s):

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### Complex Suicide, Drowning, Mechanical Asphyxia

## I12 A Massive Suicidal Ingestion of Flurazepam and Lamotrigine: A Case Report

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**Learning Overview:** After attending this presentation, attendees will better understand the main autoptotic and toxicological findings in a case of suicide due to massive ingestion of antiepileptics (flurazepam and lamotrigine).

**Impact Statement:** This presentation will impact the forensic science community by addressing the possible synergistic effect of lamotrigine and flurazepam at supratherapeutic concentrations.

A 47-year-old man with a previous diagnosis of major depressive disorder, a history of substances abuse, and multiple suicide attempts was found dead in his own house, lying on his back next to the table of the dining room. Police and rescuers found two bottles of water and an empty glass on the table and abundant (more than 60) white and blue tablets, both on the table and on the floor. They did not further specify the type of substance. In consideration of the described findings, a judicial autopsy was ordered by the prosecutor to determine the causes of death and highlight any criminal conduct. Medicolegal investigation started approximately 72 hours after the discovery of the corpse.

At the external examination, the body was 170cm in height and 110.0kg in weight. The face and clothing were diffusely smeared with abundant white, bluish, and yellowish pultaceous material from the nasal orifices and oral orifice. Minute granular formations and larger fragments referable to parts of tablets could be detected.

At the internal examination abundant cerebral edema was revealed. The opening of the airways allowed detection of abundant reddish foamy material and hyperemia of the mucous membranes; lungs were increased in weight (834g the left and 936g the right), and showed edema and vascular congestion at the examination of parenchyma. The stomach contained 280cc of whitish pultaceous material, analogous to that found on the face and clothing, with the presence of finely granular residues.

Toxicological investigations were carried out on peripheric blood, bile, urine, gastric content, and major tablet fragments found on the clothes. This research made it possible to identify the presence of lamotrigine and flurazepam in all the studied matrices. Blood concentrations of the two substances ( $>2\mu\text{g/mL}$  for flurazepam and  $>50\mu\text{g/mL}$  for lamotrigine) were above the lethal threshold described for each; positivity to benzoylecgonine was also detected in blood, urine, and bile.

Antiepileptic massive ingestion for suicidal purposes may pose serious life-threatening conditions. The use of lamotrigine as well as that of flurazepam for suicidal purposes is a rare occurrence and only rarely described in the international scientific literature; their association, on the other hand, has never been reported before.<sup>1,2</sup> In the described case, the cause of death was related to the cardiovascular effects and the depressive effects on the central nervous system, resulting from a state of acute exogenous intoxication with a possible synergistic effect of lamotrigine and flurazepam.

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### Antiepileptic Overdose, Lamotrigine, Flurazepam

## I13 Massive Sigmoid Volvulus: An Unexpected and Deadly Twist

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**Learning Overview:** After attending this presentation, attendees will be able to better appreciate the features of an unusual and life-threatening abdominal complication of some psychiatric disorders.

**Impact Statement:** This presentation will have an impact on the forensic science community by reporting the postmortem diagnosis of a massive sigmoid volvulus and secondary intestinal obstruction as a cause of unexpected death. After attending this presentation, attendees will be able to better appreciate the features of an unusual and life-threatening abdominal complication of some psychiatric disorders.

The volvulus of the sigmoid colon represents a rare condition in which an intestinal tract wraps around itself or its mesentery, resulting in closed-loop obstruction of the involved tract. In adults, it represents the third cause of the obstructive syndrome, with greater frequency in subjects suffering from comorbidities such as chronic constipation, diabetes mellitus, neurologic disorders, and previous abdominal surgery. The diagnostic suspicion is necessary when the subject presents with symptomatologic triad consisting of abdominal pain, distention, and constipation, but the clinical presentation depends on the duration and degree of colon torsion. It is necessary to carry out an early diagnosis by means of radiological and endoscopic examinations and promptly treat this condition, as it is potentially life-threatening. Indeed, complications of sigmoid volvulus include hemorrhagic infarction, perforation, septic shock, and death. The data relating to the mortality rate are not unique, but it is possible to group them in an overall range from 14% to 45%.

This report presents the unwitnessed death of a 45-year-old man, which unexpectedly occurred at his home. From the clinical history collected by the relatives, it emerged that the subject was suffering from an unspecified psychiatric pathology pharmacologically treated with olanzapine, which included obsessive behaviors such as refractoriness to defecation. Therefore, a clinical autopsy was required to establish the cause of death.

At external examination, the corpse did not show any noteworthy alterations except for abdominal distention. In the autopsy phase, at the opening of the peritoneal cavity, an unexpected and important subversion of the regular anatomy was noticed; in detail, the loops appeared extremely extended and difficult to mobilize. Analyzing the distal tract, sigma appeared considerably increased in size and rotated on its mesentery, as to describe a volvulus, with subsequent massive distension of remaining intestinal tract. Fecal content of the colon was extremely copious (895g) and presented pultaceous consistency; after abundant washing, it was possible to macroscopically identify the ischemic suffering of the wall in the absence of perforation.

In conclusion, this presentation aims to share a pathological finding rarely described in this age group by forensic pathologists who approach unexpected natural deaths. This condition, although far from usual, can be facilitated by many common conditions and must therefore be considered both by the forensic pathologist and the clinician facing a case of intestinal obstruction.

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### Sigmoid Volvulus, Bowel Obstruction, Gastrointestinal Death

## I14 Work-Related Stress Among Medicolegal Death Investigator Professionals: Sources of Stress, Health Outcomes, and Protective Factors

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**Learning Overview:** Through this session, attendees will learn: (1) how different aspects of work affect mental and physical health among Medicolegal Death Investigator (MDI) professionals, (2) the impact of COVID-19 on work-related stress and work-based supports, (3) how work-related stress varies by respondent and agency characteristics, (4) personal and work-based resources that mitigate the negative effects of work-related stress, and (5) the evidence-based recommendations for MDI professionals and their employing organizations.

**Impact Statement:** MDI professionals, including MDIs, autopsy pathologists, and forensic scientists, are routinely exposed to stressful and traumatic events and materials. This impacts their own well-being and their ability to efficiently complete their investigations, collaborate within the criminal legal system, and interact with families of decedents.<sup>1-3</sup> Yet, relatively little is known about how stress and trauma impact these professionals and how to improve their well-being. This national study is a critical first step in better understanding which aspects of work most affect MDI professionals' well-being and what individuals and organizations can do to better support the mental and physical health of this workforce. This instrument was developed in collaboration between the research team and members of the American Board of Medicolegal Death Investigators and the International Association of Coroners & Medical Examiners. The survey was fielded in April–July 2021. Over 950 MDI professionals participated in the survey. Approximately 75% of respondents identified as MDIs, but many other job roles were represented among participants, including photographer, autopsy pathologist, forensic toxicologist, and forensic analyst.

Preliminary analyses show that top stressors include dealing with distressed family members, working more than 24 hours in a row to complete work assignments, lack of understanding from political stakeholders or community leaders, and suicide among members of this workforce. Common themes from an open-ended question on sources of stress are lack of support from supervisors, inadequate compensation/resources, and lack of work breaks/time off. Prior to this session, comprehensive analysis of the survey data will be completed. This presentation will share survey findings and discuss practical implications on the following topics:

- The extent of reported work-related stress among MDI professionals and its specific sources
- Health and wellness indicators, including burnout, depression, anxiety, sleep, and post-traumatic stress disorder
- The extent to which work-related stress and work-based supports have been affected by COVID-19
- Differences in stressors and outcomes based on respondent and agency characteristics
- Personal and work-based resources for mitigating the negative effects of work-related stress, such as coping self-efficacy and organizational stress management offerings

This presentation will provide evidence-based recommendations for individuals in this workforce and their employing organizations and discuss the next phase of this project: the development, implementation, and evaluation of a stress-reduction mobile app for MDI professionals. This session will conclude with an opportunity for Q&A and interactive discussion on this critical topic of workforce health.

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### Work-Related Stress, Workforce, Medicolegal Death Investigators

## **I15 Prison Gangs, Validation, and Mental Health**

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**WITHDRAWN**

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## I16 Copping Crazy and Getting Off: What Do You Need to Know About the Insanity Defense?

George David Annas, MD, MPH\*, Forensic Psychiatry Consulting, LLC, Syracuse, NY; Corina Freitas, MD, MSc, MBA, Freitas and Associates, LLC, Baltimore, MD

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**Learning Overview:** After attending this presentation, attendees will have obtained knowledge regarding: (1) the history of the insanity defense, what it is, and what it means from a practical standpoint; and (2) the aspects of certain fact patterns of offenses that suggest a defendant has a strong case for such a defense.

**Impact Statement:** After attending this presentation, attendees will learn and/or improve skill in: (1) analyzing a defendant for an insanity defense (forensic mental health practitioners); (2) determining when to explore an insanity defense and when not to (defense attorneys); (3) determining how to approach when to prosecute a defendant who has a serious mental illness (prosecuting attorneys); (4) preserving evidence in a potential insanity case vs. other cases (Law Enforcement Officers [LEOs]); and (5) what not to do when questioning a suspect whom you believe is mentally ill (LEOs).

Despite being a part of jurisprudence since at least as far back as the second century AD, the concepts surrounding the criminal defense of “Insanity” continue to elude so many in our society. Some see it as simply an excuse for criminal behavior and others as something that mental health professionals “invented.”

This presentation will address the truth about the insanity defense from its origins in history to the modern era. Included will be the press’ and general public’s common misconceptions about the defense, as well as the reality for such defendants who are “successful” in the defense. Also addressed are the frequent misunderstandings among members of the legal profession. What are the practical aspects that prosecutors and defense attorneys need to be aware of? What pieces of evidence are important to preserve if investigators determine this will be a likely defense by the suspect?

Included in this presentation is a forensic psychiatrist who has been involved in multiple insanity cases and who will discuss the common mistakes and deficiencies among analyses within and without the forensic mental health field. This presentation will include aspects from actual cases that will serve as key teaching points for expert witnesses and attorneys, as well as LEOs.

### Reference(s):

1. AAPL Practice Guideline for Forensic Psychiatric Evaluation of Defendants Raising the Insanity Defense (Dec 2014). *Journal of the American Academy of Psychiatry and the Law Online*, 42 (4 Supplement) S3-S76. Available at: [http://jaapl.org/content/42/4\\_Supplement/S3](http://jaapl.org/content/42/4_Supplement/S3).
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**Forensic Psychiatry, Behavioral Evidence, Insanity Defense**

### I17 The Emotional State of Sexual Assault Survivors Regarding Legal Proceedings: Analysis of Connotation in Online Forums

Haruka Mori\*, George Mason University, Fairfax, VA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the psychological experiences of sexual assault survivors following legal proceedings.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from two online forums that demonstrate the emotional struggles of sexual assault survivors.

The psychological experience of sexual assault survivors is one of the most important factors to take into consideration when regarding legal proceedings. However, a systematic catalog of survivors' emotional states following legal proceedings and victim notifications has not yet been established.

Campbell et al. evaluated a victim-centered, trauma-informed victim notification protocol by categorizing survivors' reactions to notification and their decisions regarding reengagement with the criminal justice system.<sup>1</sup> They, however, did not consider the internal psychological changes of survivors. In other words, even if survivors were feeling deeply hurt on the inside, they may not have been able to express their feelings outwardly. In order to understand the internal conflicts of survivors regarding the criminal justice system, it is necessary to listen to the real voices of survivors.

In this study, qualitative text analysis of contents posted in two online public forums for sexual assault survivors was performed. Contents were extracted from two different thread categories: "Why you didn't report it" and "Status updates" starting from November 7, 2002, and June 30, 2020, to June 30, 2021. The datasets were obtained from threads of certain topics where people were most likely to discuss their experiences in victim notifications and legal procedures.

The data were analyzed using text analysis software, KH coder. The KH coder takes as inputs raw data and performs statistical analysis functionalities.<sup>2,3</sup> In this particular study, three modes of qualitative text analysis were performed: word frequency analysis, co-occurrence networks, and correspondence analysis.

Ninety-eight and three hundred twenty posts were included from "Why you didn't report it" and "Status updates," respectively. Word frequency analysis found that when sexual assault survivors discussed their experiences on the internet, they tended to use negative, trauma-based language rather than positive, forward-looking language. Co-occurrence analysis identified that negative connotative words were often associated with institutions such as hospital and counseling where survivors were likely to verbalize their experiences. Correspondence analysis identified the consistency in the writing tendencies of the survivors, which included many words associated with negative emotions.

The findings from the content analysis were that victims used negative connotations when talking about their experiences with the legal system. Words associated with negative emotions were found to be used in conjunction with other negative words such as "cry," "panic," and "afraid," clearly indicating the psychological burden in survivors of sexual assault. At the same time, it became clear that the words expressing the survivor's psychological stress were frequently used when a survivor talked about her/his own experiences and struggles regarding legal proceedings.

This present research brings to light the tremendous psychological distress brought by legal proceedings among sexual assault survivors. Further investigation of survivors' psychological impact following victim notifications about their old cases and legal proceedings is needed to avoid unnecessary re-traumatization of survivors.

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**Sexual Assault, Forensic Medical Examination, Criminal Justice System**



## I18 Suicide Trends in Adults and COVID-19: A Retrospective Analysis in Georgia

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**Learning Overview:** Attendees will increase their understanding of how adult suicides trends have fluctuated during the COVID-19 pandemic in both urban and rural communities. This will inform attendees for the purposes of disaster response efforts, policy development, and resource allocation to better serve vulnerable populations.

**Impact Statement:** The goal of this study is to emphasize the critical role of forensic science during times of crisis, particularly amid workforce shortages and resource limitations. As government and public health agencies seek to address emerging threats, increasing awareness of how disaster and economic downturns impact mortality has become increasingly important during the era of COVID-19.

The onset of the COVID-19 pandemic affected every facet of life in the United States, including suicide rates. As the tenth-leading cause of death nationwide, suicide continues to pose a substantial public health threat to high-risk populations.<sup>1</sup> This presentation demonstrates how the mental health of urban and rural communities has been impacted by the pandemic and, consequently, the corresponding effect this has had on the medical examiner/coroner system in the state of Georgia.

This study examined suicide cases involving decedents ages 18 years or older during a two-year period between 2019 and 2020. In accordance with the Georgia Death Investigation Act (O.C.G.A. § 45-16-24), these cases were investigated by the appropriate medical examiner or coroner's offices in DeKalb, Hall, Henry, Rockdale, and White counties.

Despite strict isolation and quarantine measures implemented in the city of Atlanta, urban suicide rates decreased by 30.9% over the course of the pandemic. Comparatively, rural suicide rates remained relatively constant amid relaxed COVID-19 restrictions. This disparity could be attributed to factors such as lower socioeconomic status, mental health care stigmatization, and fewer resources in rural areas.<sup>2</sup> Suicide impulsivity, indicated by the absence of a suicide note or disclosure of intent, increased by 14.1% in urban areas and decreased by 21.0% in rural areas. Despite a nationwide increase in firearm sales during the height of COVID-19, urban firearm-related suicides decreased 41.1% with a corresponding 8.0% increase in firearm-related homicides.<sup>3</sup> While both groups experienced an increase in suicides involving intimate partner problems, urban suicides tended to involve financial and job problems, whereas rural suicides involved health-related problems (most commonly cancer and chronic pain). While the pandemic presented the opportunity for many individuals to strengthen their interpersonal relationships, COVID-19 exacerbated relationship decline in those experiencing disproportionate chronic stress, which may have contributed to the increased incidence of intimate partner-related suicide.<sup>4</sup> In urban areas with lockdown-induced closures, the most substantial increase in suicides occurred in the food and beverage industry (83.3%), while sales and government experienced notable declines (87.9% and 72.7%, respectively). In rural areas, decedents in the entertainment, education, and construction industries were more likely to commit suicide throughout the pandemic, although no particular industry experienced an increase in suicides.

In the setting of the COVID-19 pandemic, this study explores the implications of prolonged stress and how suicide mortality reflects health care disparities in urban and rural communities.

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### Forensic Pathology, Suicide, COVID-19

## I19 Outpatients' Social Reintegration in the Community in Italy After High Security Hospitals Close

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of the reunification and reintegration of psychiatric services after Law 81/2014 and the possibility of their collaboration with other social agencies is intended to end the stigma that is attached to the criminal justice system and also to raise the standard of care.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from the treatment for socially dangerous mentally ill people and marginalized people in need of psychiatric treatment that should respect their rights and dignity and should be aimed to their reintegration.

The Law Number 81 May 30, 2014, that established in Italy one or more Residences for the Execution of Security Measures (REMS) as replacements for High Security Hospitals (Ospedale Psichiatrico Giudiziario [OPGs]) in each region of the country for socially dangerous Not Guilty by Reason of Insanity (NGRI) offenders, has followed after more than 40 years the closure of psychiatric hospitals (Ospedale Psichiatrico, OP). A unique community following the public therapeutic model supported the reintegration of all mentally ill patients, offenders or not, back into their family and society under the auspices of the Department of Mental Health (Dipartimento di Salute Mentale, DSM).<sup>1</sup> A REMS is a residential community that is integrated into the larger community model of general psychiatry.<sup>2</sup> By December 2020, there were 620 inpatients in Italy's 31 active REMSs, 80 of whom were women.<sup>3</sup> A REMS is an exceptional measure, which must end as soon as possible and provide care and security to those subjects who suffered from a severe mental illness at the time of the crime who did not have criminal responsibility or had diminished criminal responsibility, but present a high level of social danger. The analysis on REMS therapeutic activities demonstrated a combination of pharmacological, rehabilitative, and psychotherapeutic treatments.<sup>3</sup> With regard to the reintegration of outpatients with mental illness who have committed crimes, this study took into consideration the reality of the Puglia region (region is the first administrative body of the state) from January 2018 to December 2018. In Puglia (four million inhabitants), there are two REMS (38 beds total), but currently there are also seven forensic facilities (residential communities dedicated to forensic inpatients, [CRAP dedicata]) with ten beds each with another 70 forensic inpatients on conditional release (Libertà Vigilata). The median length of stay of inpatient treatment was 11.3 months in REMSs. After discharge from the REMS, some patients continue the treatment in the forensic communities on conditional release for longer periods (median length of stay, 22.7 months). Women occupied 10–12% of the regional secure beds. In 84.6% of cases, all samples committed a crime against a person and 15.4% a crime against property. Common diagnoses were schizophrenia (42.3%) and other psychotic spectrum disorders (30.7%) with bipolar disorder (7.7%) cases. Substance use disorder was present in 15.4% of all cases.

The municipalities have made themselves available to a project in collaboration with the health services for the social and occupational recovery of forensic outpatients. The municipalities can promote some socio-work recovery projects for the mentally ill in collaboration with the mental health services and in the same way can proceed for patients up to 50 years of age discharged from REMS and forensic facilities, selected by the DUNDRUM Toolkit 4 currently being validated in Italy.

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### REMS, DUNDRUM, Social Reintegration

## I20 DUNDRUM: An Italian Validation Project

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of risk assessment and needs assessment for therapeutic security in an Italian forensic treatment model through a structured professional judgment based on the use of internationally validated scales and tools.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence from the predictive assessment of the level of therapeutic safety for patients with mental disorders who are offenders at risk of violence and recidivism in the Italian forensic context, profoundly changed after Law 81/2014.

The DUNDRUM Toolkit is an assessment tool consisting of four different scales (Structured Professional Judgment Tools for Admission, Urgency, Treatment Completion, and Recovery Evaluation Triage) used with forensic patients for evaluation and treatment purposes.<sup>1</sup>

The DUNDRUM, like other tools such as the HCR-20, also recently validated in Italy, among the most used in the literature for assessing the risk of violence and recidivism in convicted subjects, is commonly used for Structured Professional Judgment (SPJ).<sup>2,3</sup> SPJ is increasingly recognized as an effective way to improve the quality, consistency, and transparency of the decision-making process regarding the safe treatment of patients with mental disorders who committed crimes and are at risk of violent acts.

Considering the utility of this tool, which is flexible and broad in its scope of application, especially in a forensic treatment model such as the Italian one aimed at rehabilitation, it lends itself well to the assessment of the level of therapeutic safety and security for forensic patients with mental disorders and for planning treatment and the assessment of treatment appropriateness and effectiveness.

This original tool was made available to the mental health departments that coordinate Residences of Execution of Security Measures (REMS), the forensic facilities that replaced high-security hospitals in Italy after their closing, as well as forensic psychiatrists and researchers validating it for the Italian population and the Italian health system.<sup>4</sup> For the validation of the DUNDRUM toolkit in Italy, following translation, a preliminary phase was carried out aimed at training the researchers involved. A person specially trained in Dublin at the Central Mental Hospital where the instrument was developed has provided training through a series of monthly meetings for a total of five meetings for the representatives of Italian REMS and other forensic facilities.

In the next phase, the largest possible number of patients included in the Italian REMS was enrolled out of the total of over 600 hospitalized, after obtaining their written consent to the research.<sup>5</sup> For the purpose of validation, it was expected to enroll no less than 200 patients. At the same time, a series of anamnestic, clinical, and criminological data was acquired through the compilation by the researchers of a specially constructed form. Periodic checks on the progress of patient enrollment were also provided through comparison with all researchers involved. The research was approved by the Ethics Committee of the University Hospital of Bari. All data will be processed by the group of researchers who designed the tool.<sup>6</sup> The first research data will be presented at the American Academy of Forensic Sciences (AAFS) conference.

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### Mental Illness, Forensic Contexts, Risk of Violence/Recidivism

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## 121 The Differences Between Readmitted and Non-Readmitted Women in an Italian Forensic Unit: A Retrospective Study

*Ilaria Rossetto, MD, PhD\*, Sistema Polimodulare di REMS Provvisorie, Castiglione delle Stiviere, Italy*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of the ongoing research on individual risk and protective factors as well as risk assessment tools on conditionally and unconditionally released female inpatients and the value of studies that consider genders separately.

**Impact Statement:** This presentation will impact the forensic science community by presenting the variables linked with readmission in Italian forensic psychiatric services for socially dangerous mentally ill women.

The main objective of this study was to compare Readmitted (RW) and Non-Readmitted (NRW) female psychiatric patients after being conditionally or unconditionally released from Italian inpatient forensic psychiatry services in order to identify variables that were significantly linked with readmission.

This study included all patients who were discharged from the female Residences for the Execution of the Security Measure (REMS) of Castiglione delle Stiviere from January 2008 to June 2015 who were not readmitted until December 31, 2018. In addition, data were collected on female patients who were discharged from the same REMS before 2008 and readmitted from January 2008 and December 2018. A key finding of this study was that the readmission into a female REMS was positively associated with the presence of Substance Use Disorders (SUD) and a primary diagnosis on Axis II. To a lesser extent, younger age, being unconditionally discharged when first released, having had a shorter length of inpatient stay, and having committed a crime against property for the first REMS admission were also variables that were apparently linked with readmission. The present research continues the previous research on gender-specific mentally ill offenders.<sup>1</sup> Hence, the decision was made to proceed separately with a sample of men-only offenders and another of women-only offenders. For all these reasons, young female patients with personality disorders and substance use disorders should perhaps remain longer in REMS and be released with conditions. In most European countries, the length of stay depends on the clinical condition and risk assessment, with some exceptions when the courts set a maximum length of stay at the outset, as in Italy.<sup>2</sup> All factors listed above influence the risk assessment.<sup>3</sup>

Finally, from integrating these findings into the increasing international literature on conditional release and considering the recent changes in the Italian forensic treatment model, this study recommends continuing research on individual risk and protective factors as well as risk assessment instruments on conditionally and unconditionally released inpatients with genders studied separately.<sup>4</sup>

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### Discharge, Female Forensic Psychiatry Inmates, Readmitted

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## I22 A Psychopath in the House: How Digital Media Is Used to Exploit Even the Safest Homes

*Jessica Morel, DO\*, Fayetteville, NC; Vivian Shnaidman, MD, Princeton, NJ; Anna-Renee Labak, Enfield, CT*

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**Learning Overview:** The goal of this presentation is to discuss various ways in which digital media enters into forensic psychiatric evaluations and also how digital media investigations may uncover forensic psychiatric issues. Through updates on current crime statistics and various case discussions, this presentation will explore how digital media can be used to exploit the mentally ill as well as how psychopaths and sociopaths may use digital media to find victims.

**Impact Statement:** This presentation is designed to help the forensic sciences community understand the interface with digital media and forensic psychiatry. As technology continues to develop, the mentally ill are finding victims online and becoming victims to predators. Forensic evaluators are discovering elements of digital media influence in their case assessments and litigation.

Digital media is a growing forensic field as technology is frequently changing and expanding. Society used to assume that their homes were where they were safest. However, as criminals find new ways to exploit technology, investigations are leading back to the home. Cases may be presenting with more and more evidence that encompasses the expertise of forensic psychiatry just as digital media is affecting psychiatric treatment on a daily basis. Oftentimes, the digital media investigators and psychiatrists do not have the opportunity to interface on these cases and the two interpretations of data meet in the court room.

Many in society do not understand the psychological underpinnings of sociopaths and psychopaths, with their similarities and distinctions, nor do they separate these offenders from other individuals with mental health diagnoses that are more likely to be victims. With society turning more and more to technology and social media for personal interactions, we are unwittingly inviting perpetrators into our homes and personal lives. Young adults in particular may have superior skills in using technology, but they lack judgement and are often impulsive, resulting in exploitation. Older adults, on the other hand, can be naïve to the ways in which digital media may be used to attain personal information and exploit finances. With more recent isolation of society due to COVID-19 lockdowns, digital media investigations are becoming more relevant than ever. Potential victims are going online to find interpersonal connections they once found at work and in social activities. Meanwhile, perpetrators are finding and developing new platforms to access their victims at home.

Equally important to acknowledge is the very important evidence that digital media may provide when the perpetrator is deceased or at large. We are starting to see cases play out in the news before they hit the court room due to the wide availability of some digital media platforms such as social media pages, security cameras, and text messages. This presentation will also address the negative impacts that misinterpretations have on cases due to the ease of digital media editing or selection bias by news stations. It is important that we as investigators bear in mind that our first impression may not encompass the whole picture, and we should remain vigilant in our investigations to explore the data further. Digital media data investigations are an asset to our cases, and we should ensure the dissection of this information through thorough psychological interviews and criminal investigations where possible.

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### Psychopath, Sociopath, Digital Media

## I23 Virtual Reality as a Treatment Medium for Sex Offenders

Jacob Pourat, MD\*, USC Institute of Psychiatry and Law, Los Angeles, CA

**Learning Overview:** After attending this presentation, attendees will: (1) understand how Virtual Reality (VR) is currently being utilized in the field of mental health care, and (2) be able to critically appraise the potential uses, benefits, and limitations in using VR in sex offender treatment.

**Impact Statement:** This presentation will impact the forensic science community by comparing traditional forms of assessing and treating deviant sexual interests with new and theoretical VR methodologies.

VR is a computer-generated simulation that allows people to interact with a 3D and seemingly real virtual environment. VR has been recently introduced into medicine, where it is currently being used for both training and treatment. For example, VR is now being utilized to treat patients with neuromuscular conditions by simulating fun and engaging activities to increase engagement with physical therapy. In the mental health field, VR is more commonly utilized to provide simulated exposure therapies for post-traumatic stress disorder, anxiety disorders, and specific phobias.<sup>1,2</sup> More recently, VR technology is gaining recognition in forensic mental health with the potential to be more prevalent as a treatment tool.<sup>3</sup>

When looking at objective measures in sex offender treatment, a substantial body of research shows that deviant sexual interest is a key element in explaining sexual offending behaviors and one of the strongest risk factors for sexual reoffending.<sup>4,5</sup> This in turn places immense importance on accurately assessing sexual interest for treatment aimed at reducing the risk of reoffending. Penile Plethysmography (PPG), which measures blood flow to the penis as a proxy for sexual arousal, is often used for identification of deviant sexual interest. PPG is used by exposing people to sexually arousing stimuli, most commonly 2D pictures and/or sounds. In one study, researchers found that VR has the potential to improve on the ability to identify deviant sexual interests compared to other tools that use simpler imagery.<sup>6,7</sup>

VR may enhance the current state of sexual offender treatment. VR has the capability to place sex offenders in different, customizable environments that resemble real-life scenarios and settings (e.g., beaches, parks, grocery stores) while simulating specific interactions with virtual characters. For example, a person who is known to be attracted to minors could be placed in a simulated park to assess how they react to the presence of virtual children. VR can also be combined with other tools commonly used for identifying sexual interest, such as PPG.<sup>8</sup> As a treatment medium, VR has the potential to be used in psychotherapy with the goal of teaching self-identification of deviant cognitions, self-regulation of behavior, and relapse prevention. Furthermore, by using 3D simulated characters, VR circumvents the ethical and legal issues posed by using real imagery to elicit sexual arousal.<sup>3</sup> Even though there are multiple potential benefits of using VR in the forensic setting, it is also important to consider its limitations, such as the lack of research and standardization in using VR in forensic treatment and the high initial cost to incorporate VR for treatment purposes.

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### Virtual Reality, Sex Offender, Treatment

## I24 From Online Personas to Mass Murderers: The Incel Ideology and the Struggles of Mental Health

*Eva Ip, BS\*, British Columbia Institute of Technology, Burnaby, British Columbia, Canada*

**Learning Overview:** The learning objectives of this presentation are for attendees have a better understanding of what an involuntary celibate (incel) is, how they have developed into an extremist group over the years, and why there needs to be a push for more research to be conducted on this community.

**Impact Statement:** This presentation aims to impact the forensic science community by presenting research that was collected from inside incel web-based forums, which was able to produce unexpected but astonishing results.

Incels are individuals who are part of a virtual community of isolated men who do not have a sexual life and collectively see women as the cause to their problem of celibacy.<sup>1</sup> In the online communities they frequent, topics of feminism and women are often subjected to hostility and violent threats.

There have been multiple instances throughout history where incels have executed attacks to gain a sense of notoriety and postmortem fame among their online communities; an infamous example was Elliot Rodger, a 22-year-old man who committed a mass murder in Isla Vista in 2014, where he killed 6 individuals and injured 13.<sup>2</sup> Rodger stated that he committed this act as a response to the hardship he faced in losing his virginity. Sexual shame is often associated with traditional masculinity, heavily suggesting that misogynistic men are more likely to be vulnerable to risk factors. The increased rate of suicide deaths, psychosis, violence, and incarceration in men is significantly higher when they experience this shame.<sup>3</sup> However, little research has been conducted on the mental health aspect of an incel and the role that it plays leading up to mass attacks toward the greater population.

This research investigated whether there are underlying mental health factors that contribute to an incel's antagonism toward women and if it ultimately leads to them committing a mass attack. The study applied an exploratory research design as it used secondary research from various academic databases and open-source queries. The data that was collected focused on using web-based discussion boards from Reddit.com and Incels.net in which eight variables were coded. The posts and the comments corresponding were observed over a six-month time frame to reduce any potential bias from the researcher.

The results indicated a strong connection between mental health factors and the antagonism an incel feels toward women and demonstrates where future research could build on what was found to strengthen that connection.

### Reference(s):

1. Sylvia Jaki; Tom De Smedt; Maja Gwózdź; Rudresh Panchal; Alexander Rossa; and Guy De Pauw. Online Hatred of Women in the Incels.me Forum. *Journal of Language Aggression and Conflict* 7, no.2 (July 2019): 240-268, <https://doi.org/10.1075/jlac.00026.jak>.
2. Peter Langman. Elliot Rodger: An Analysis. *Journal of Campus Behavioural Intervention* 2, (November 2014): 5-19, 10.17732/JBIT2014/1.
3. James L. Knoll IV; George D. Annas. Mass Shootings and Mental Illness. In: *Gun Violence and Mental Illness*, eds. Liza H. Gold and Robert I. Simon (American Psychiatric Publishing, 2015), 81-104.

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### Group Aggression, Extremism, Mental Health

## I25 3D Interactive Lineups Improve Eyewitness ID Accuracy

Heather Flowe, PhD\*, University of Birmingham, Edgbaston, United Kingdom

**Learning Overview:** After attending this presentation, attendees can expect to learn: (1) the key lineup procedures that are in use worldwide to identify criminal suspects; (2) key memory theories concerning eyewitness identification; and (3) the latest research on 3D interactive lineups, which significantly increase the identification of guilty suspects and reduce the identification of innocent suspects.

**Impact Statement:** The forensic science community will be impacted by learning about the potential for new and low-cost technology to improve criminal investigations.

In many countries (including the United States), criminal identification lineups consist of static photographs. Lineup members are shown from the shoulders up, facing forward, even if a witness viewed the perpetrator from a different angle at the time of the crime (e.g., profile view). This study tested whether a novel interactive lineup procedure increases identification accuracy compared to traditional lineup procedures. This study also examined whether witnesses would spontaneously rotate the lineup faces to match the angle in which they saw the perpetrator and, if so, whether this is associated with increased identification accuracy. In tests conducted with 3,000 participant witnesses, this study found that identification accuracy, the ability to differentiate guilty from innocent suspects, is significantly increased when witnesses can view the lineup members at the same angle as they saw the perpetrator commit the crime.<sup>1</sup> Moreover, this study found in tests with 10,000 participants from around the world that identification accuracy is significantly increased compared to the traditional static frontal pose lineups.<sup>2</sup> Interactive lineups also increase accuracy in other race identifications compared to traditional procedures. Faces are 1.4 times more likely to be misidentified if they are of a different race than the person making the identification and own race bias is a leading cause of erroneous eyewitness identification. These errors are reduced significantly in interactive lineups. Interactive lineups are proving to be a valuable tool for increasing identification accuracy. Interactivity has also been found to boost forensic face-matching accuracy, in both typical as well as superior face recognizers.<sup>3</sup> More information about research on interactive lineups can be found in the latest issue of *Scientific American*.<sup>4</sup>

### Reference(s):

1. Colloff, M.; Seale-Carlisle, T.M.; Karoğlu, N.; Rockey, J.C.; Smith, H.M.J.; Smith, L.; Maltby, J.; Flowe, H.D. (In press). Criminal identification viewed from a different angle: Enabling witnesses to reinstate perpetrator pose during a lineup test increases accuracy. *Scientific Reports*.
2. Colloff, M.F.; Flowe, H.D.; Smith, H.J.; Seale-Carlisle, T.M.; Meissner, C.A.; Rockey, J.C.; Pande, B.; Kujur, P.; Parveen, N.; Chandel, P.; Singh, M.M.; Pradhan, S.; Parganiha, A. (In press). Active exploration of faces in police lineups increases discrimination accuracy for own- and other-race faces. *American Psychologist*.
3. Smith, H.M.J.; Andrews, S.; Baguley, T.S.; Colloff, M.F.; Davis, J.P.; White, D.; Flowe, H.D. (In press). Performance of Typical and Superior Face Recognisers on a Novel Interactive Face Matching Procedure. *British Journal of Psychology*.
4. Digital Heads Help Eyewitnesses Identify Suspects. *Scientific American*.

### Eyewitness, Lineup, Identification



## J1 The Department of Justice Uniform Language for Testimony and Reports (ULTR) For Forensic Document Examination (FDE)

*Carl R. McClary MS\*, U.S. Department of Justice, ATF, Atlanta, GA*

**Learning Overview:** Attendees will learn what the Questioned Document sections of Department of Justice (DOJ) forensic science laboratories have published to be used as reporting and testimony language. Attendees will also gather ideas for terms and definitions for their own opinions on common source.

**Impact Statement:** This presentation will impact the forensic science community by providing the most updated information on what the DOJ has done to standardize testimony and reporting language across its forensic laboratories. Attendees will learn what, specifically, the examiners within the DOJ Questioned Document (QD) community are currently using to communicate the results of examinations within the discipline. Attendees will learn the five primary conclusions, with bases and reasons included, that are being utilized within the QD sections. These conclusions are based on previously published standardized opinion terminology found within the Scientific Working Group of Forensic Document Examiners (SWGDOC) standard T01-13.

The DOJ has developed and published standardized opinion language for various disciplines within its forensic science laboratories. These laboratories reside in the following agencies: Drug Enforcement Agency (DEA), the Federal Bureau of Investigation (FBI), and the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF). These documents are provided as guidance for scientific statements when drafting reports or testifying. Currently, 14 disciplines have approved language for reporting and testimony.

Representatives from both DOJ laboratories offering Forensic Document Examination (FDE) services collaborated on a QD working group that convened over a period of two years, meeting both in person and virtually, to gain consensus on the number of terms and definitions used in describing FDE opinions. Because the primary sub-examination of FDE is handwriting and hand printing comparisons, the scheme of opinion levels focuses on accommodating these examinations. However, other examinations, such as paper fracture match, typewriting, and hand stamp (rubber stamp) examinations, can be applicable to the ULTRs. The nine-level standardized opinion terminology widely used served as the basis for the working group. The process also took into consideration language from Bayesian processes, current disparate levels and language in use in the two laboratories, and standardized ULTRs from other disciplines. The FDE ULTR was published on July 5, 2021.

The final product of the working group, below, reflects an easily usable compilation of opinion levels (terms) providing five primary opinions to cover the circumstances of many FDE examinations. Within the two qualified levels is language that can be expanded, if necessary, to reflect the strength of those qualified terms.

**Source identification** (i.e., identified)—an examiner’s conclusion that two or more bodies of writing were prepared by the same writer. The examiner would not expect to see the same combination of characteristics repeated in a body of writing by another writer.

**Support for common source**—an examiner’s conclusion that two or more bodies of writing may have been prepared by the same writer.

**Inconclusive**—an examiner’s opinion that no determination can be reached as to whether two or more bodies of writing were prepared by the same writer or by different writers.

**Support for different sources**—an examiner’s conclusion that two or more bodies of writing may not have been prepared by the same writer.

**Source exclusion** (i.e., excluded)—an examiner’s conclusion that two or more bodies of writing were not prepared by the same writer. Bodies of writing exhibit different handwriting characteristics and no significant limitations or circumstances, such as an alternative writing style or skill level.

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### Questioned Document Examination, ULTR, Common Source

## J2 Classification of Handwriting Using Pen Pressure Patterns

*Yoko Seki MA\*, National Research Institute of Police Science, Kashiwa, Chiba, Japan*

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**Learning Overview:** After attending this presentation, attendees will understand the effectiveness of pen pressure data acquired online in the classification of handwriting.

**Impact Statement:** This presentation will impact the forensic science community by presenting the experimental procedures and the classification results of handwriting classification experiments using online data.

The development of information technology has greatly changed our ordinary lives. The environment surrounding writing and writing materials has changed greatly. Writing with a finger or a stylus pen on a tablet instead of writing with a pen on paper, especially in the case of signing, has become more and more popular.

The number of forensic handwriting examination cases in which handwritten evidence is acquired online has been increasing, along with the widespread use of various online writing systems. As such, the necessity for the development of forensic handwriting examination methods for online data processing has also grown.

In this presentation, visualization and synthesis of dynamic information on writing acquired online were attempted using a “pen pressure map,” where the pen pressure change pattern was expressed on the pen tip trajectory in three colors. A Convolutional Neural Network was used for writer classification experiments with online and offline data.

Handwriting classification experiments using LeNet were conducted. Online and offline data of four categories of Kanji characters written by ten Japanese adults five times each were used for the experiments. Three samples per writer per category were used for the training data, and the remaining two samples were used for test data. Four categories of characters were combined in a fixed order to be a Japanese person’s name, and the character string of the name was used for the experiments. Any sample of the same category of the same writer in either training or test data was combined following the fixed order. So, 81 (the fourth power of three) samples of training data and 16 (the fourth power of two) of test data were obtained per writer. Online data were processed as follows: online image data of each sample were acquired by drawing the pen tip trajectory; the pen pressure value data were processed using the pen pressure change between two measuring points; and the pen pressure change data between the two points were expressed on the trajectory in three colors, that is, blue (pen pressure increasing), yellow (pen pressure unchanged), and red (pen pressure decreasing). Classification experiments were done on both online and offline data.

Classification results showed greater than 0.95 accuracy, precision, recall and F-measure scores obtained for both online and offline data. Nearly the same correct ratio was obtained in handwriting classification using both online and offline data. This suggests the possibility of handwriting classification using samples written on a tablet may be the direction of future handwriting identification.

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**Handwriting Classification, Pen Pressure, Convolutional Neural Network**

### J3 Contemporary Studies Via Chemometric and Spectroscopic Techniques for the Investigation of Questioned Documents

Vishal Sharma, PhD\*, Institute of Forensic Science & Criminology, Panjab University, Chandigarh, UT, India

**Learning Overview:** The detection of written as well as computer-generated document forgeries has always been a challenging task for Forensic Document Examiners (FDEs). With the aim of supporting the examination processes, Diffuse Reflectance Spectroscopy Ultraviolet Visible (DRS-UV-Vis) Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR), and Schottky Field Emission-Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (FE-SEM/EDS) are explored as tools to analyze handwritten ink as well as toner-printed documents. Various handwritten and laser-printed documents from various sources were produced and analyzed through these analytical techniques.

**Impact Statement:** This research will be useful for FDEs as it provides objective results, and most of the methods are non-destructive in nature. Moreover, the chemometric methods increase statistical confidence in the results, which is highly desired in this field. Moreover, in the future, it is quite possible to create a database of additional inks and toner samples for the identification of unknown inks.

The techniques used in present day analysis of handwritten documents include UV-Vis, ATR/FTIR, and FE-SEM/EDS. In the present research, handwritten and laser-printed documents obtained from various sources were analyzed.<sup>1,2</sup> The obtained spectral data was then used for applying chemometric models for their classification, discrimination, and characterization.<sup>3</sup> The samples from the laser printer and photocopier machines were also studied for their morphological features, elemental profile, and multivariate analysis. The evaluation of SEM images and spectra allowed for discrimination among the samples and classification of toners as having different sources of origin. The application of multivariate analysis was conducted for developing a model of classification for both inks as well as toner samples solely based upon their similarities and dissimilarities in composition. Hierarchical cluster analysis discriminated ink and toner samples in the form of groups based on their chemical makeup. Further cross-validation was conducted for assessing the capabilities of developed Principal Component Analysis (PCA) and linear discriminant analysis models for the examination of inks and toners from an unknown origin.

#### Reference(s):

1. Neha Verma, Raj Kumar, Vishal Sharma. Analysis of laser printer and photocopier toners by spectral properties and chemometrics. *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 196 (2018) 40.
2. Vishal Sharma, Raj Kumar. Fourier transform infrared spectroscopy and high performance thin layer chromatography for characterization and multivariate discrimination of blue ballpoint pen ink for forensic applications. *Vibrational Spectroscopy* 92 (2017) 96-104.
3. Raj Kumar and Vishal Sharma. Chemometrics in forensic science. *TrAC Trends Anal. Chem.* 105 (2018) 91-201.

#### Toner, Chemometric, Questioned Documents

## J4 Indentations Developed on Three Reams of Paper Using an Electrostatic Detection Device (EDD)

Rafal K. Turek, MS, IRS, Chicago, IL; Martin Shlaymoon, PhD\*, IRS, Chicago, IL

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the possible origin of the indentations that can be made on paper before it reaches the user.

**Impact Statement:** This presentation will impact the forensic science community by presenting the marks and other features created on paper during the paper manufacturing process.

Repetitive indentation patterns were developed with the EDD on reams of 8.5" x 11" paper from the same manufacturer. Three different brands of paper were sampled.

The indentations that appeared on these reams included:

- A pair of scuff marks on the front of the first sheet of each ream.
- Diagonal lines appeared on the front of the first and fifth sheets of two of the reams, and parallel waves appeared on the third ream.
- A mirror image of the diagonal lines appeared on the reverse of the fifth and sixth sheets of two of the reams, and a mirror image of the parallel waves appeared on the third ream.

The above indentations repeat throughout the ream every n+6 sheets, indicating that the ream has been comprised of paper from six different rolls during the sheeting process. There were scuff marks on sheets of each ream, but the appearance of the marks (the size, shape, and distance between them) are what differed from ream to ream. Each of the examined reams of paper consisted of 504 sheets (not 500 as appeared on the label), which is consistent with the fact that the ream is made up of six rolls (because 504 is divisible by 6, but 500 is not).

These indentations are assumed to have been produced during the paper manufacturing and conversion process, where larger rolls are reduced in size and assembled into reams. The differences suggest they could be produced at different plants or mills or under different machinery configurations.

This presentation should make examiners aware that indentations can occur not only from the handling of a document by a user, or from the business machines used in its printing, scanning, or copying, but also from the paper stock itself, created during the manufacturing process. This presentation will show the indentations found and attempt to explain their likely sources.

### Reference(s):

1. International Paper Co. (1997). *Pocket pal: A graphic arts production handbook*. (17th ed.).
2. LaPorte, G. The Use of an Electrostatic Detection Device to Identify Individual and Class Characteristics on Documents Produced by Printers and Copiers—A Preliminary Study. *Journal of Forensic Sciences*, 2004, 49(3).
3. Olson, L. Indentations Produced by the Document Feeder Mechanisms of Two Black and White Photocopiers. *Journal of the American Society of Questioned Document Examiners*, 2009, 12(1): pp. 1-18.
4. Ellen, D., Foster, D. and Morantz, D. The Use of Electrostatic Imaging in the Detection of Indented Impressions. *Forensic Science International*, 1980, 15(1): pp. 53-60.

### Indentation , Paper Examination, EDD

## J5 Assessing the Dependency Structure Between Shape Codes for Forensic Handwriting Data

Cami Fuglsby, MS\*, South Dakota State University, Brookings, SD; Kayla Moquin, BS, FBI Laboratory | RSU, Quantico, VA; Christopher Saunders, PhD, South Dakota State University, Brookings, SD; Danica Ommen, PhD, Iowa State University, Ames, IA; Michael Caligiuri, PhD, University of California, San Diego, La Jolla, CA; JoAnn Buscaglia, PhD, FBI Laboratory | RSU, Quantico, VA

**Learning Overview:** Attendees will gain an understanding of how to compare certain feature sets stemming from the output of an automated handwriting identification system and how the comparison relates to the dependencies between features of handwritten characters.

**Impact Statement:** This presentation will impact the forensic science community by investigating how certain handwriting features correlate and how those features interact with a scoring method. This may have an impact on future studies on automated handwriting data pertaining to score development or assumptions on the amount of information contained within a handwritten document.

Automated handwriting identification systems typically use a complex set of algorithms to extract the feature sets from a given handwriting sample. For example, in the original CEDAR-FOX system, the features could be grouped into macro- and micro-feature sets.<sup>1,2</sup> Other systems, such as the FLASH ID<sup>®</sup> and Handwriter, tend to extract different sets of features based on the orientation of the segmented writing.<sup>3,4</sup> For the FLASH ID<sup>®</sup> system, the orientations of segmented writing are referred to as shape codes. In recent work, a set of pairwise scoring rules, known as the Vector-Of-Counts (VOC) method, has been developed that can be decomposed across the shape codes that have been observed in a given pair of documents.<sup>5</sup> An important aspect of understanding how the FLASH ID<sup>®</sup> system works for pairwise comparisons is to find the relationship between shape code-specific VOCs and their corresponding correlations with an omnibus VOC. In effect, the greater the correlation between the subcomponents of the omnibus score, the less effect adding in the second subcomponent will have on the omnibus score; ideally, the omnibus score will be constructed in terms of independent subcomponents so that, in any given comparison, there is an understanding of why a given score is observed.

To study this relationship, a class of new statistical regression techniques developed in Caligiuri, and extended in Tang, will be used to provide a statistically rigorous characterization of the dependency between different VOC-shape code pairwise similarity scores.<sup>6,7</sup> These new statistical techniques are based on using least squares methods on the set of all pairwise comparisons between writers and account for the new dependency structure between the comparisons.

The results of the regression models will be explained and presented in terms of the original observed writing samples with a focus on the graphical display of the shape codes, score development, and resulting findings.

### Reference(s):

1. Srihari, S.N., Srinivasan, H., Desai, K. Questioned document examination using CEDAR-FOX. *J Forensic Document Examination*, 2007; 18:1-20.
2. Srihari, S.N., Cha, S.H., Arora, H., Lee, S. (2002). Individuality of handwriting. *J. forensic Sci.* 47 1-17.
3. Sciometrics, LLC, Chantilly, VA, USA. <https://www.sciometrics.com/flashid.html>
4. Berry, N., Taylor, J., Baez-Santiago, F. (2021). handwriter: Handwriting Analysis in R. *R package version 1.0.1* <https://CRAN.R-project.org/package=handwriter>.
5. Fuglsby, C., Saunders, C., Ommen, D., Buscaglia, J., Caligiuri, M. Elucidating the relationships between two automated handwriting feature quantification systems for multiple pairwise comparison. Manuscript submitted to *Journal of Forensic Sciences*.
6. Caligiuri, M. Kinematic Validation of FDE Determinations about Writership in Questioned Handprinting and Handwriting. *National Institute of Justice, 2019. Report No.: 2017-DN-BX-0148*.
7. Tang, L. Accounting for Covariates in Forensic Error Rate. *National Institute of Justice, 2019. Report No.: 2019-DU-BX-4011*.

### Handwriting, Automated Identification Systems, Dependency Structures

## J6 Standards Development Activities Related to Forensic Document Examination

*Kevin P. Kulbacki, MS\*, KDX Forensic Consulting, LLC, Chicago, IL*

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**Learning Overview:** After attending this presentation, attendees will better understand the status of discipline-specific standards pertinent to the field of forensic document examination.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities related to document examination. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, standards development updates related to forensic document examination will be discussed. These include: (1) OSAC proposed standards (Scope for Expertise in Forensic Document Examination, Standard for Forensic Examination of Handwritten Items, Forensic Document Examination Terms and Reference, Standard for the Preservation and Examination of Charred Documents, and Standard for the Examination of Liquid Soaked Documents); (2) published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process (Standard for the Examination of Documents for Alterations, Standard for the Examination of Documents for Indentations, and Standard for the Examination of Stamping Devices and Stamp Impressions); and (3) documents currently in development at the OSAC or by the ASB (Standard for Minimum Training Requirements for Forensic Document Examiners, Standard for the Examination of Mechanical Checkwriters, Standard Guide for Initial Evidence Assessment of Questioned Document Evidence, Standard for the Expression of Conclusions in Handwriting Evidence, Standard Test Methods for the Comparison of Writing and Inkjet Inks, Standard for the Examination of Dry Seals, and Standard for the Collection for Known Writing), and other highlights. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### Questioned Documents, Standards, OSAC

## J7 The Accuracy and Reliability of Forensic Handwriting Examiner Decisions

Linda Eisenhart BS, MBA\*, FBI Laboratory, Quantico, VA; R. Austin Hicklin, PhD, Noblis, Reston, VA; Brian Eckenrode, PhD, FBI Laboratory, Quantico, VA; Peter J. Belcastro, Jr., MA, FBI Laboratory, Quantico, VA; Ted M. Burkes, BS, FBI Laboratory, Quantico, VA; Connie L. Parks, MA; Noblis, Reston, VA; Meredith DeKalb Miller, MFS, Chicago, IL; Joann Buscaglia, PhD, FBI Laboratory, Quantico, VA; Michael A. Smith, PhD, FBI Laboratory, Quantico, VA

**Learning Overview:** Attendees will learn about the results of a large-scale Handwriting Decision Analysis (Black Box) Study, including accuracy, error rates, reproducibility, and repeatability of handwriting decisions and what factors impact these rates.

**Impact Statement:** This presentation will enable forensic document examiners to answer questions regarding what factors impact the accuracy and reproducibility of the decisions, including those related to the examiner and those related to the samples.

This Handwriting Decision Analysis (also known as Black Box) Study was designed and conducted by the Federal Bureau of Investigation (FBI) Laboratory, Ideal Innovations, and Noblis. The design of this study was based on the FBI Laboratory-Noblis latent print examiner black box study, and in consideration of comments made in two United States Reports: the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Pathway Forward* and the 2016 Report from the President’s Council of Advisors on Science & Technology (PCAST) titled *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*.<sup>1-3</sup>

Participation for this study was limited to practicing handwriting examiners who had performed casework within the past two years. A pre-test survey was completed by each participant to gather information about their education, training, experience, certification, and day-to-day work. Participants were asked to perform a total of 100 comparisons of paired handwriting samples, each containing one sample of questioned writing and up to five pages of writing from a single known subject. In an effort to represent the wide range of evidence encountered in casework, a diversity of handwriting samples was selected. The test samples included a range of styles of handwriting (i.e., cursive, hand printing, and mixed), content (e.g., addresses, a few sentences, predefined London or CEDAR letters, or freeform text), known writing samples containing the same or different wording, as well as varying amounts of questioned and known writing. Note that this study was limited to the comparison of handwritten documents and did not include the comparisons of signatures. Great effort was taken to provide participants with test packets that were relatively challenging, both for mated and non-mated pairs.

Using an online platform, participants were provided with high-resolution digital images that could be downloaded for comparison. The participants used this platform to respond to predefined questions that included the conclusion, limitation(s), assessments of comparability and variation within questioned and known writing samples, perceived difficulty of the comparison, and most influential characteristics that were used in making their decisions. Participants were given five conclusions to choose from, including definitive opinions (written by and not written by), qualified opinions (probably written by and probably not written by), and no conclusion. The study resulted in 7,213 trials from 86 handwriting examiners on 180 distinct comparisons of questioned and known handwriting.

This presentation will provide the results of the study, including the accuracy, reproducibility (inter-examiner variation), and repeatability (intra-examiner variation) of decisions made by forensic handwriting examiners. This presentation will expand upon the results provided at the 2021 AAFS meeting by presenting the factors that did (or did not) impact the accuracy of the decisions in this study, including those related to the examiner (such as training or experience) and those related to the samples (such as quantity of writing, comparability of content, limitations, or style of writing).

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1. Ulery, Bradford T., R. Austin Hicklin, JoAnn Buscaglia, and Maria Antonia Roberts. 2011. Accuracy and reliability of forensic latent fingerprint decisions. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*.
2. 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.
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.

### Handwriting, Error Rate, Black Box Study

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## J8 Expert Disclosure Requirements in the Federal Rules of Civil Procedure and the Federal Rules of Criminal Procedure

*Jan Seaman Kelly, BA\*, Forensic Dynamics LLC, Las Vegas, NV*

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**Learning Overview:** Attendees will have a clear understanding of the differences between Rule 26 in the Federal Rules of Civil Procedure and Rule 16 in the Federal Rules of Criminal Procedure and the impact of the 1993 revisions that were designed to expedite the litigation process.

**Impact Statement:** This presentation educates the forensic science community on the differences between Rule 26 of the Federal Rules of Civil Procedure and Rule 16 of the Federal Rules of Criminal Procedure. Understanding the requirements that Rule 16 and Rule 26 place on expert witnesses highlights the importance of meeting the requirements, since the failure to do so will result in exclusion of that portion of the witness testimony.

Rule 26 of the Federal Rules of Evidence Civil Procedure was revised and went into effect on December 1, 1993. This revision was controversial as it required parties in a lawsuit to exchange certain information at the beginning of the litigation, including the disclosure of the expert's written report. The Supreme Court's intent for Rule 26 was to encourage openness between the involved parties during the early stages of the dispute as a means to improve the efficiency of the federal judicial process. The Court's intent was three-fold: (1) early discovery benefits both sides to focus on the discovery needed and to facilitate trial or settlement preparation; (2) assists the parties to determine whether or not depositions will be necessary; and (3) requires the parties to share information as well as have a clearer idea of document requests. These three benefits were designed to avoid disputes between attorneys and to accelerate the exchange of basic information. Compliance with Rule 26 in the Federal Rules of Civil Procedure occurs when the expert's written report has been disclosed.

Compliance with Rule 16 in the Federal Rules of Criminal Procedure is not as laborious as Rule 26 in the Federal Rules of Civil Procedure. Under Rule 16, the expert is not required to draft nor to submit a report prior to testifying in court. However, the expert is required to provide a written summary of testimony that includes the issued opinion, the basis and reasoning for each opinion, and the qualifications of the witness.<sup>1-4</sup>

Attendees will have a clearer understanding of the differences between Rule 26 in the Federal Rules of Civil Procedure and Rule 16 in the Federal Rules of Criminal Procedure and the impact of the 1993 revisions designed to expedite the litigation process.

**Reference(s):**

1. Mervis, Michael T., Esq., Hawthorne, Kelly Landers, Esq. Expert testimony without an expert report. *Westlaw Today*, November 12, 2020.
2. Federal Rules of Evidence Rule 26.
3. Federal Rules of Evidence rule 17.
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### Rule 26, Rule 16, Litigant



## J9 Institutional Prejudice by the Courts Involving Certain Opposing Handwriting Opinions

*Thomas W. Vastrick, BS\*, Apopka, FL; Jan Seaman Kelly, BA, Forensic Dynamics LLC, Las Vegas, NV*

**Learning Overview:** After attending this presentation, attendees will be aware of certain prejudices held by triers of fact against indeterminate and highly qualified opinions. In addition, attendees will learn practices that can mitigate these prejudices.

**Impact Statement:** This presentation will bring to light a recognized concern of inequitable consideration by courts of highly qualified or indeterminate conclusions. This inequity can conceivably result in “incorrect decisions on culpability or liability,” as noted by the Forensic Handwriting Examination and Human Factors report.<sup>1</sup> It is distinctly hoped that this presentation will contribute to a lesser chance of such injustice within our court system.

The discipline of forensic document examination in the United States has primarily utilized a series of opinion terms since an effort to standardize terms was first introduced many years ago. This presentation delves into user reaction to instances in which there are opposing opinions of varied qualifications/confidence levels/likelihoods. There have been instances in which differing opinions have been misrepresented in litigation and indeterminate opinions have been the source of minimization or outright mockery. Discussions among examiners show that it has become apparent that this problem is far reaching.

In 2016, a study of forensic document examiners who have provided indeterminate opinion testimony in court was conducted. Forty-seven forensic document examiners from around the world responded to the survey, noting that testimony of indeterminate opinions was provided in 519 instances: 245 in criminal court, 178 in civil court, 1 United Nations tribunal, 1 immigration hearing, 12 military hearings, 62 juvenile hearings, 8 bench trials, 2 administrative hearings, 3 *Daubert* hearings, and 7 depositions. In 103 of these instances, an indeterminate opinion was the sole opinion provided.

There are multiple reasons why a forensic document examiner appropriately provides indeterminate opinions. Oftentimes, these reasons include severe examination limitations or conflicting data in which neither associative nor dissociative support is compelling.

Concern has arisen as to how indeterminate and highly qualified opinions are viewed in court. One reference, quoted in the Forensic Handwriting Examination and Human Factors report from the National Institute of Standards and Technology (NIST) states, “The findings suggest that jurors tend to over-value some attributes of forensic science expert testimony and under-value other aspects. The most persistent finding is that jurors rely heavily on the ‘experience’ of the testifying expert and the expert’s asserted certainty in conclusions.”<sup>1</sup> The Human Factors report also notes two additional studies supporting that jurors prefer unambiguous testimony that is strongly worded, ultimately providing this ominous warning, “These findings are a concern because if jurors and others give greater credence to strong opinions that might not be as well reasoned or well founded as more complex, qualified opinions, they may make incorrect decisions of culpability or liability.”<sup>1</sup>

There are steps that can be taken by the forensic document examiner to counter this bias. These steps include: (1) clearly denote qualifications; (2) detail the methodologies used and directly denote the industry standard reference step by step; detail, with images, the limitations imposed on the examination; (4) provide the raw data in detail; (5) address alternative hypotheses in as much detail as the initial opinion; and (6) provide substantial detail in an easy-to-understand court presentation, complete with proper illustrations.

### Reference(s):

- <sup>1</sup> Expert 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. 2020. NISTIR 8282. Published February 18, 2020.

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### Opinions, Handwriting, Courts

## J10 The Manual Reconstruction of Shredded Documents

*Meg OBrien, MS\*, USSS, Washington, DC*

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the basic steps of reconstructing shredded document evidence, as well as the time-consuming nature of these cases.

**Impact Statement:** This presentation will impact the forensic science community by presenting cases involving shredded documents outside the normal scope of paper documents with machine-printed or handwritten text.

Shredded documents are collected from various places and in different types of investigations. The manual reconstruction of shredded documents is a time-consuming process. Imagine being provided multiple jigsaw puzzles, then told to combine all the pieces into one pile, throw away the boxes, and then put the puzzles together. That is essentially how a shredded document case is worked.

The basic steps of a shredded documents case are: (1) sorting, and (2) reconstructing. Sorting is performed based on characteristics such as paper color, directionality of shred, presence of handwriting or machine printing, and font style and color, among other features. Reconstruction of documents can be hindered if multiple versions or copies of documents are included.

The goal of reconstructing shredded documents is to provide information for investigators. This information is needed in a timely manner, but the reconstruction of shredded documents is a time-consuming project. The quantity and size of shred are just two factors that can affect the time needed by the forensic document examiner. An additional factor that affects how long a case takes includes a primarily remote work schedule while evidence must remain in a secure facility, such as a laboratory.

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### Questioned Documents, Shredded Documents, Document Reconstruction

## J11 Electrostatic Detection Apparatus (ESDA): Something Old, Something New

*Larry A. Olson, MFS\*, IRS National Forensic Laboratory, Chicago, IL*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding about: (1) what indentations artifacts may occur when using the ESDA2 or ESDA2/B, and (2) how to use the ESDA2 for examining bound materials.

**Impact Statement:** This presentation will impact the forensic science community by: (1) reminding document examiners how business machines may cause indentations on paper documents, (2) revealing that ESDA artifacts/defects may cause what appear to be indentations, and (3) reintroducing some possibly long-forgotten instructions from the original ESDA device.

This presentation is in two parts. The first part, Something New, deals with the ESDA2 or ESDA2/B creating artifacts that were mistaken for indentations on papers being examined. In recent years, this study had developed a specific pattern of voids (six columns of white dots) in the ESDA lifts of documents examined in several fraud investigation cases. The most obvious common denominator in the cases was that prior to the submission of the documents, the investigators had made copies using the Automated Document Feeder (ADF) on their copiers. The same pattern occurred on documents passed through the ADF of the laboratory's copier (because the agency had a service-wide contract for the same make and model). After much research and exhaustive correspondence with the copier manufacturer, the origin of the pattern appeared to point to the copy imaging process, where a bar of LED lights in two columns (roughly the same dimensions as the voids) illuminated the document as it passed through the ADF. The other (unconscious) common denominator, however, was that it was the lab's ESDA2 and ESDA2/B that were used in developing the indentations. It was found that a colleague at another lab had experienced the same phenomenon, which turned out to have been caused by an excessive vacuum being pulled through the ESDA platen.

Another instance of an ESDA2 artifact being mistaken for indentations on a document was the result of the softness of the metal used in the platen. In another lab, the spine of a bound notebook being examined embossed the ESDA platen, and the embossing was reproduced on ESDA lifts and initially assumed to be indentations from a document.

The second part of this presentation, Something Old, is a revisiting of possibly forgotten procedures on examining bound books for indentations. These procedures were described in the original ESDA operating instructions but were not included in manuals for the ESDA2.<sup>1,2</sup> One method requires placing the entire book on the platen with a sheet of metal foil under the sheet being examined to conduct the electric charge. Another method involves positioning the book against the instrument so that only one sheet is on the platen. Younger examiners who have never worked with the original ESDA may be unaware of these methods, although they work equally well on the ESDA2 or 2/B and are, additionally, non-destructive to the book.

**Reference(s):**

1. ESDA Operating Instructions, Foster & Freeman Ltd., undated.
2. ESDA-2 Electrostatic Detection Apparatus User Manual 11-03, Foster & Freeman Ltd., undated.

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**Indentation, ESDA, EDD**

### **J12 Exploring Digital Capture and Comparison of Indented Writing Developed With an Electrostatic Detection Device**

*Mark Goff, BA\*, Michigan State Police, Lansing, MI*

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**Learning Overview:** This presentation evaluates various methods of digitally capturing writing indentations developed using an Electrostatic Detection Device (EDD), the subsequent comparison of these images to the source writing to show source attribution, and an overview of the applicable areas of the American National Standards Institute/ Academy Standards Board (ANSI/ASB) Standard 044, Standard for Examination of Documents for Indentations, First Edition, 2019.

**Impact Statement:** After attending this presentation, attendees will have an understanding of the options available for the digital capture and comparison of writing impressions developed with an EDD, methods for conducting comparisons of the digitally captured images, and the applicable current Forensic Document Examination standard.

Multiple methods of digital image capture were tested with varying degrees of success. These methods included: scanning the developed indentations on the platen of an EDD with an inverted Contact Imaging Sensor (CIS) flatbed scanner; a Charge-Coupled Device (CCD) flatbed scanner; and digital photography, using a 22 mega pixel digital camera and a 50 mega pixel digital camera. These imaging methods were compared to physical lifts of the same developed indentations. Scanning with a CCD scanner and digital photography showed results comparable to indentions preserved with a physical lift. Photography of EDD-developed writing indentations showed an increase in efficiency for the forensic document examiner when compared to the time and effort related to the physical encapsulation of the developed image and production of an EDD lift.

The second half of this presentation will provide an overview of the methods of comparison for digital images of EDD-developed indentations to source writing with a raster graphics editing program. This presentation will conclude with an overview of the applicable standards and how they relate to the digital capture and comparison of EDD-developed indentations.

The digital capture and comparison method proved to be a quick and viable way to show source attribution for writing impressions developed with an EDD.

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#### **EDD, FDE, Standards**

## **J13 An Objective Inter-Comparison of Trash Mark Constellations From 50 Photocopiers Utilizing Manual and Automated Detection Methods**

*Linda Eisenhart, BS, MBA\*, FBI Laboratory, Quantico, VA; Joseph C. Stephens, MFSF, FBI Laboratory, Quantico, VA; Jocelyn V. Abonamah, MFS, District of Columbia Office of the Chief Medical Examiner, Washington, DC; Paige Riley, ORISE/FBI Laboratory, Quantico, VA; Brian Eckenrode, PhD, FBI Laboratory, Quantico, VA; Colbey Ryman, Noblis, Arlington, VA*

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**Learning Overview:** The goal of this presentation is to demonstrate the foundational validity of trash mark comparison methods through empirical, objective data. By harnessing this objective data, forensic scientists can define and apply mathematical calculations and statistical approaches to make the analysis more transparent and reproducible.

**Impact Statement:** This presentation will impact the forensic science community by outlining a method to move trash mark examinations toward pre-defined decision criteria and data analyses. This presentation will detail the results of an inter-comparison of the trash mark constellations from 50 known photocopiers using a novel, objective method. This method also provides an improvement in efficiency, especially when numerous known machines with multiple functions are being compared to one or more questioned documents.

Trash marks are individual characteristics observed on printed, scanned, or photocopied documents that result from permanent or transient defects on the glass platen, slit glass, photosensitive drum, or lid of office machines. When multiple trash marks resulting from the platen are present, a pattern—or constellation—can be recognized and used for source attribution of questioned documents. Trash mark examinations have been employed in forensic laboratories for decades, yet the method remains relatively untested and relies on training, experience, and anecdotal information to support its validity. This study generated and harnessed objective data to empirically test one of the foundational theories for assessing source attribution of photocopied documents: if trash marks are present in sufficient quantity and/or quality, no two machines will exhibit a constellation of trash marks that is indiscriminate from another. In this study, objective trash mark location and size data was generated for 50 known photocopiers using both a traditional and a novel automated detection method. Intermachine comparisons were conducted using a custom variant of the Hausdorff distance to generate a quantitative assessment of how similar or different the 2,450 pairs of trash mark constellations from this study were. This presentation will report the results of the inter-comparison of the trash mark constellations of 50 photocopiers using both traditional and automated detection methods.

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### **Defect Marks, Photocopy, Printing**

## J14 Barcodes: Basics and Cases

*Carolyn Bayer-Broring, MFS\*, Homeland Security Investigations Forensic Laboratory, McLean, VA; Elaine Wooton, MFS, Homeland Security Investigations Forensic Laboratory, McLean, VA*

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**Learning Overview:** Barcodes appear in the casework of forensic document examiners in a variety of forms and formats. The forensic document examiner needs to be prepared not only to understand the purpose a barcode serves on the document being examined, but also how to examine the barcode, make minor repairs to damaged barcodes, and extract the information necessary to assist in assessing the legitimacy of that document.

**Impact Statement:** By attending this presentation, attendees will have an understanding of the purpose and function of barcodes on documents and will be able to explain the outcomes of examinations in which barcodes are interrogated.

Barcodes are ubiquitous and, for the most, part innocuous. However, the use of barcodes in security documents brings the assessment of questionable versions under the purview of the forensic document examiner. Fortunately, equipment developed for laboratory examination of documents includes software to decode barcodes. This allows examiners to undertake the most common activity regarding barcodes, which is determining if the information in a barcode matches the information printed on a security document.

While the software allows for assessments without a lot of knowledge about the type of barcode being assessed, examiners should have a basic knowledge of barcodes so they can describe them and explain the agreement or disagreement between the barcode and the printed information. Basic expertise in this area includes knowledge of the history of barcodes, various types of barcodes, uses for barcodes, as well as barriers or issues with barcode interrogation.

A brief historical overview includes the initial use of barcodes for tracking rail cars, followed by grocery scanning, inventory tracking (commercial and military), and, finally, the widespread use of various barcode versions, including the best known—the QR code.

The simplest way to categorize barcodes is as “1D” or “2D.” 1D barcodes are simple and typically hold a very small amount of data. They are formulated by converting numbers or characters into the elements of a barcode font. Individuals who work with 1D barcodes can often read them as if they were written in their native language. 1D barcodes may contain a short inventory control number or point to an inventory system. 2D barcodes, such as the PDF-417, Data Matrix, and QR code, can contain extensive information depending on the number of rows and columns they include. They are encoded with complex software and incorporate various levels of error correction, so they can be decoded even if they are damaged.

To date, there have been a handful of cases at the laboratory in which barcodes played a significant role. A summary of those cases will be presented and include: identification of fraudulent forms due to the presence of identical 1D barcodes, reconstruction of damaged PDF-417 and Code 39 barcodes, and documentation of mismatches between postal barcodes and correspondence.

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### Barcodes, PDF-417, QR Code

## J15 Forensic Methods to Detect the Presence of Hidden Messages

*Kirsten Tate, BS\*, Federal Bureau of Investigation, Quantico, VA*

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**Learning Overview:** Attendees will learn about methods of concealing a secret message in a written letter, as well as methods of identifying these hidden messages.

**Impact Statement:** This presentation impacts the forensic science community by bringing awareness to the existence of secret messages that are difficult to detect and by introducing research on a new approach to detecting hidden messages in a larger body of text.

People who commit crimes have plenty of reason to share secrets, and they often resort to various forms of secret writing to accomplish this. The field of forensic cryptanalysis seeks to identify and break these encoded messages in order to reveal their true meaning. *Concealments* are one such method often used by criminals to exchange secret messages without attracting the attention of law enforcement. In a letter-based concealment, the letters of a secret message are systematically scattered throughout a larger body of text. The cover text might be something seemingly innocuous, but the intended recipient of the message is able to extract the correct letters to spell out the secret message.<sup>1</sup> It can sometimes be difficult for a forensic cryptanalyst to determine whether a concealment is present in a given message, since it is hidden within this larger body of “normal” text. However, it has been observed anecdotally that writers of concealments typically write in a different style than they would otherwise. Indeed, recent research has shown that when a person is writing a concealment, their writing style does in fact change from the writer’s natural style. In addition, this phenomenon can be quantified using techniques from a field of statistics called authorship attribution.<sup>2</sup>

A recent study showed that writers tend to change their sentence length significantly when writing concealments, and they also tend to use different vocabulary and sentence structure than they would otherwise. This novel result can be used to compare a suspected concealment against other writing samples from the same author in order to determine whether a concealment is likely to be present.<sup>3</sup> This development provides forensic cryptanalysts with a new tool to use when analyzing a possible concealment. This presentation will introduce concealments as a method of secret writing and show how authorship attribution can be used to identify them.

**Reference(s):**

- <sup>1</sup> Kahn, D. 1996. *The codebreakers: The comprehensive history of secret communication from ancient times to the Internet*. New York: Scribner.
- <sup>2</sup> Stamatatos, E. 2009. A survey of modern authorship attribution methods. *Journal of the American Society for Information Science and Technology* 60(3): 538-556.
- <sup>3</sup> Tate, K. 2021. Identifying concealed messages using authorship attribution. *Cryptologia* 45(2): 167-177.

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**Cryptanalysis, Secret Writing, Statistics**

## J16 The Forensic Document Examiner Forum

*Karen Nobles, MFS\*, Forensic Document Services, LLX, Pensacola, FL; Carl R. McClary, MS U.S. Department of Justice, ATF, Atlanta, GA; Samiah Ibrahim, BSc, BA, Canada Border Services Agency, Ottawa, Ontario, Canada*

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**Learning Overview:** After attending this presentation, attendees will have access to new and different ideas, opinions, and methods to consider when approaching questioned document problems in the laboratory. These ideas and methods will represent experiences from different countries and cultures.

**Impact Statement:** The impact of this presentation on the forensic science community will be the open exchange of ideas and viewpoints. As scientists, we often learn as much from another's thought processes and experiences as we do from books. The younger examiners can benefit from the experiences of the more-experienced examiners, and those experienced examiners can benefit from seeing new and novel viewpoints.

The concept of the Forensic Document Examiner Forum is not new to the Questioned Document community. This format was first utilized at the Southeastern Association of Forensic Document Examiners (SAFDE) meetings in the early 1990s as a method of opening discussion on certain topics of interest to the members. At these meetings, there was a discussion starter on subjects upon which there may have been disparate opinions. Through forums of this type, there have been many lively discussions on controversial topics. Not all discussions must necessarily be centered on difficult issues facing forensic document examiners. Discussions may also focus on the attendee's thoughts and interpretations of quotes from recognized texts in the field. Other discussions could begin with the conclusions and opinions of some of these famous authors in criminal and civil cases of notoriety, both past and present. This type of forum, one which is presented with international viewpoints, is an excellent way to poll attendees about their preferences on examination methods, conclusions, court testimony, or the current standards in the field.

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### Discussion, Methods, Conclusions



### J17 A Type of Forgery in a Forensic Document Recently Seen in Turkey: A Case Report

*Dilara Oner, PhD\*, Istanbul, Turkey; Berna Senel Eraslan, Istanbul University-Cerrahpasa, Istanbul, Turkey; Gursel Cetin, PhD, Istanbul University-Cerrahpasa, Istanbul, Istanbul, Turkey*

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**Learning Overview:** This presentation will ensure that attendees are informed about a method of preparing forged documents that is frequently used today and that is likely to become more widespread in the future.

**Impact Statement:** Though forensic document examiners may know that a genuine signature from one document can be placed on another document for fraudulent purposes, this method of forgery may not be encountered often in casework. This presentation will demonstrate a case where this method of forgery was used and how it was detected.

A common form of forgery in recent years is to have a person sign a fake receipt for cargo that has been received through a fake distributor. The person signs in a “window-shaped” section with the rest of the document concealed. When the front paper is later removed, a blank paper with the signature on the bottom is revealed for the criminal to insert a deed or similar text to make the signer financially liable.

In the presented case, expert witnesses were asked by the court whether the signatures on a questioned deed belonged to the debtor. The signatures belonged to the debtor; however, it was determined that the promissory note was not the original promissory note paper. There was a 1mm difference between the right and left edges, the lower edge was not a preprinted cut, and there were traces of glue in a rectangular shape around the debtor’s signature. In this case, the plaintiff debtor claimed that the deed was fake, and his petition mentioned an unexpected, suspicious parcel in the form of a gift. This background information helped to guide the examination to include the whole document, rather than just a signature comparison.

The presented case has a twofold significance. First, it is necessary for the public to know that such a form of forgery exists and to pay attention to what is being signed, especially when receiving unexpected merchandise. Second, it reminds the expert to consider and examine the whole document.

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**Forgery, Forensic Questioned Document Examination, Forged Promissory Note**

## J18 Fraudulent Backdating and Writing Sequence in Questioned Documents

*Mohamed Awadalla, PhD\*, Forensic Lab, RAK Police, Ras Al Khaymah, UAE*

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**Learning Overview:** After attending this presentation, attendees will have discovered an explanation for how to systematically study the problems of falsely given dates of documents and forged writing sequences.

**Impact Statement:** This presentation will impact the forensic science community by introducing a comprehensive study of one of the problems facing Forensic Documents Examiners (FDEs) and inform attendees as to how to find solutions to these problems.

Sequential backdating of a document is considered an important type of examination conducted by an FDE. Documents can be backdated to make them appear as though they were issued or signed earlier; thus, the examiner must attempt to determine the sequence of preparation of the document. The signature may be genuine, but the date was altered. This study classified factors that affect sequential dating into two main approaches: static and dynamic. The static approach includes all stable factors, meaning those facts that cannot be changed and relate to the document's history, such as writing ink, writing instruments, stamps, and paper. The dynamic approach addresses changeable factors and are subdivided into factors related to the state of the writer at the time and those related to the document condition. This presentation will review sample cases from casework as illustrations and will present a recommendation regarding examinations.

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**Document Backdating, Writing Tools, Writing Circumstances**

## J19 Forensic Handwriting Examination Education and Training Curriculum Development: Making the Ideal Real

Mara Merlino, PhD\*, Kentucky State University, Frankfort, KY; Veronica B. Dahir, PhD, University of Nevada-Reno, Reno, NV; Charles Edwards, MA, University of Nevada-Reno, Reno, NV

**Learning Overview:** Attendees will learn how to apply academic concepts and methods to create education and training programs that incorporate best practices identified by stakeholder organizations such as the National Institute of Standards and Technology (NIST), Organization of Scientific Area Committees (OSAC), and Forensic Science Education Programs Accreditation Commission (FEPAC). Attendees will participate in applying these concepts to the education and training program format demonstrated in the 2021 NIST publication titled *Forensic Handwriting Examination and Human Factors: Improving the Practice Through a Systems Approach*.<sup>1</sup>

**Impact Statement:** This presentation will impact the field of Questioned Document Examination by applying a multidisciplinary approach, including academic and practitioner perspectives, to the task of creating a standardized training program as recommended by stakeholders such as NIST, OSAC, FEPAC, and other relevant stakeholder organizations.

Over the past 20 years, stakeholders in the forensic science enterprise have been engaged in discussions about the reliability and validity of the methods and procedures used by forensic practitioners. These discussions have included recommendations for the content of forensic science education programs, calls for standardized and empirically based education and training based on academic models, and recommendations and examples of the format and content of such education programs.

This presentation will be an interactive demonstration and discussion of how these goals may be addressed through interdisciplinary collaborations between subject matter experts in education and curriculum development and professional document examiners. According to FEPAC, the training program structure should delineate learning objectives, instructor qualifications, requirements for students, and performance goals. Periodic assessments and competency testing should be included. The training program syllabus should be detailed enough to ensure that all these components are clearly understandable and empirically measurable.

Core elements of training programs should include both ethics training and standards of conduct; training in effective verbal and non-verbal communication skills, with attention to evidence handling, the legal environment, and rules of evidence; laboratory policies and operating procedures; and safety practices. Some suggested discipline-specific elements include relevant literature to impart key knowledge about this history of the discipline and the current state of knowledge in the field, including validation studies for methods and procedures, statistics, and training on instrumentation.

These topics will be discussed in the context of the education and training model published in the 2021 publication titled *Forensic Handwriting Examination and Human Factors: Improving the Practice Through a Systems Approach*.

Attendees will engage with presenters in deconstructing these broad elements of a training and education program and suggesting possible program goals, learning outcomes, and benchmarks for demonstrating trainee competence.

### Reference(s):

- <sup>1</sup> Taylor, M., Bishop, B., Burkes, T., Caligiuri, M., Found, B., Bird, C., Grose, W., Logan, L., Melson, K., Merlino, M., Miller, L., Mohammed, L., Morris, J.J., Osborne, N., Ostrum, B., Saunders, C., Shappell, S.H., Srihari, S., Stoel, R., Vastrick, T., Waltke, H. and Will, E. (2021). *Forensic Handwriting Examination and Human Factors: Improving the Practice Through a Systems Approach*. NIST Interagency/Internal Report (NISTIR), National Institute of Standards and Technology, Gaithersburg, MD, [online], <https://doi.org/10.6028/NIST.IR.8282r1>, [https://tsapps.nist.gov/publication/get\\_pdf.cfm?pub\\_id=932534](https://tsapps.nist.gov/publication/get_pdf.cfm?pub_id=932534) (Accessed November 5, 2021).

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### Training, Education, Questioned Documents

## J20 Revealing the Deposition Order of Optically Indistinguishable Blue Ballpoint Pens Using Mega Electron-Volt-Secondary Ion Mass Spectrometry (MeV-SIMS) Coupled With Multivariate Analysis

Marko Barac, MSc\*, Ruđer Bošković Institute, Zagreb, Croatia; Andrijana Filko, MSc, Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia; Zdravko Siketić, PhD, Ruđer Bošković Institute, Zagreb, Croatia; Marko Brajković, MSc, Ruđer Bošković Institute, Zagreb, Croatia; Andrea Ledić, MSc, Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia; Iva Bogdanović Radović, PhD, Ruđer Bošković Institute, Zagreb, Croatia

**Learning Overview:** After attending this presentation, attendees will be acquainted with the capabilities of an accelerator-based mass spectrometry technique called MeV-SIMS, which is unconventional in the context of forensic questioned document examination.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the power of solving a problematic case with MeV-SIMS in the determination of deposition order of two very similar ballpoint pens when the standard optical techniques fail.

Today, most official documents, such as contracts or testaments, which are often the subject of counterfeiting, are produced with commercially available printers. On such documents, a stamp impression or signatures using different types of pens are also applied. The places where such writing tools intersect may reveal if the document is original or was modified in any way after the moment it was produced. Various techniques have been introduced in forensic work to solve the problem of intersecting lines. A comprehensive review of different methods that have been used so far was given in the work of e Brito et al.<sup>1</sup> It was emphasized that optical methods that are non-destructive, fast, and easy to apply will probably remain prevalent in the daily forensic work and that the need for other analytical techniques, which are more expensive and complicated to perform, sometimes destructive and time consuming, would appear in more complex cases where the information obtained from optical methods is inconclusive and not sufficient to give a proper answer.

Recently, accelerator facilities around the world have started to use heavy energetic ions for excitation of secondary molecular ions from the sample surface—an Ion Beam Analysis (IBA) technique called MeV-SIMS. In the field of forensic examination of questioned documents, the technique has demonstrated success in determining the deposition order of fingerprints and inks on paper.<sup>2</sup> In the case of intersecting lines made by blue ballpoint pens, the deposition order of all studied combinations was determined with success.<sup>3</sup> The situation was not so clear in some cases when deposition order of inkjet inks in combination with other writing tools was studied.<sup>4</sup> Here, some cases were solved by combining MeV-SIMS with the Particle-Induced X-ray Emission (PIXE) technique which, contrary to surface-sensitive MeV-SIMS, provides information from deeper layers.

In the present work, intersections of several very similar ballpoint pens were studied using optical non-destructive techniques (microscopic and infrared luminescence) that are standardly applied for questioned documents examination in the Forensic Science Centre "Ivan Vučetić" and MeV-SIMS that is applied at the Ruđer Bošković Institute. Obtained results were compared and discussed. An emphasis is placed on the ability of MeV-SIMS in combination with multivariate analysis to distinguish two very similar ballpoint pen inks and determine their deposition order. These cases appear to be problematic to Forensic Science Centre "Ivan Vučetić" using any optical method at hand. MeV-SIMS also provides chemical information about the studied writing tools, which is an added value and can be also applied to differentiate the two writing tools in question.

### Reference(s):

1. E Brito, L.R., Martins, A.R., Braz, A., Chaves, A.B., Braga, J.W., Pimentel, M.F. Critical review and trends in forensic investigations of crossing ink lines. *TrAC Trends in Analytical Chemistry* 2017, 94, 54-69.
2. Bailey, M.J., Jones, B.N., Hinder, S., Watts, J., Bleay, S., Webb, R.P. Depth profiling of fingerprint and ink signals by SIMS and MeV SIMS. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 2010, 268 (11-12), 1929-1932.
3. Malloy, M.C., Bogdanović Radović, I., Siketić, Z., Jakšić, M. Determination of deposition order of blue ballpoint pen lines by MeV Secondary Ion Mass Spectrometry. *Forensic Chemistry* 2018, 7, 75-80.
4. Moore, K.L., Barac, M., Brajković, M., Bailey, M.J., Siketić, Z., Bogdanović Radović, I. Determination of Deposition Order of Toners, Inkjet Inks, and Blue Ballpoint Pen Combining MeV-Secondary Ion Mass Spectrometry and Particle Induced X-ray Emission. *Analytical Chemistry* 2019, 91 (20), 12997-13005.

### Forensic Document Examination, Optical Techniques, MeV-SIMS

### J21 Hyperspectral Imaging and Gray Value Analysis of Porous-Tipped Pen Samples Composed of Unknown Inks

*Clarra Moore BS\*, Sam Houston State University, Huntsville, TX; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX*

**Learning Overview:** After attending this presentation, attendees will understand how to apply gray value analysis and hyperspectral imaging to the analysis of porous-tipped pen ink strokes and blended ink samples.

**Impact Statement:** This presentation will impact the forensic science community by providing questioned document examiners with an objective, non-destructive image analysis method to analyze porous-tipped pen samples.

Porous-tipped pens are a low-researched area within questioned documents. Analytical methods used to analyze porous-tipped pen samples range from video spectral comparisons to thin-layer chromatography to Raman spectroscopy. Chemical analytical methods typically require destructive sample preparation. Image analysis is an objective approach that can provide numerical information without damaging the document. Hyperspectral Imaging (HSI) and gray value measurements are two such methods. HSI can be achieved with a simple Video Spectral Comparator (VSC). Images are obtained using a range of wavelengths and are then stacked to create a data cube. A spectrometer is then used to obtain the spectral information from each pixel within the 3D data cube. Thus far, HSI has been used to discriminate between ballpoint and gel ink samples and has proven to be an advantageous addition to visual examination of pen inks. While there is little information on the application of HSI to porous-tipped pens, the art history and conservationist field commonly uses HSI to identify colorant components of historically or culturally significant documents and pieces of art. Gray value measurement analysis has much less research. Applying the program ImageJ, images obtained using Infrared (IR) filters can be further analyzed more objectively than solely using visual examination. IR absorbance and luminescence of pen inks are useful characteristics for discrimination between samples, and gray value measurement is an additional application that should be further explored. A section of the image is selected in the software, and each column is an average of the grayscale values, or the amount of light on a scale from 0 to 255, from each row. Gray value analysis has been found to be useful for discriminating between porous-tipped pen ink stroke samples; however, analysis for blended samples needed further improvement.<sup>1</sup>

This current project aims to propose an improved method of gray value analysis that has been established by Moore and Buzzini, while also incorporating HSI to the analysis of unknown porous-tipped pen ink stroke and blended ink samples.<sup>1</sup> The goal of this project is to use gray value analysis and HSI to determine the composition of unknown porous-tipped pen samples. A volunteer was given access to a collection of porous-tipped pens and instructed to write the same statement three times using three different inks of their choosing. This volunteer was also instructed to create a blended ink sample of their own choosing using up to six pens of their choosing. These blind samples were then compared with porous-tipped pen standard samples created on Whatman® filter paper using visual examination, gray value analysis, and HSI. Based on the visual examination, the hypothesized standard pen ink samples were used for further comparisons. Images were obtained using eight different light filters of the VSC. Images obtained using IR absorbance and luminescence were further analyzed using gray values analysis in ImageJ. HSI reflectance spectra was obtained using the software provided by the VSC instrument used.

**Reference(s):**

- <sup>1</sup> Moore, C., Buzzini, P. Analysis of colored felt-tipped blending marker ink using gray value measurements of visual spectral data. *Proceedings of the 77<sup>th</sup> Annual General Meeting of the American Society of Questioned Document Examiners*; 2019 August; Raleigh, NC, U.S.

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**Questioned Documents, Ink Analysis, Image Analysis**

## J22 Indented Writing Examinations: Rubber Stamp Image Transfers

*James A. Green, BS\*, Eugene, OR*

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**Learning Overview:** Attendees will learn about unusual rubber stamp images recovered with the use of an electrostatic detection device.

**Impact Statement:** The information presented will make the forensic document examiner community aware of a potential misinterpretation of evidence recovered with the use of an electrostatic detection device.

Electrostatic detection devices, used by forensic document examiners, are well known for their ability to recover indentations from documents. The impressions are usually the result of the pressure generated from pen strokes written upon a document placed above another document. However, images are occasionally captured that are not simply the result of pen pressure, but are due to other types of paper fiber disturbances.

This presentation is related to an uncommon type of non-impact indented writing recovered during casework. An indentation image was recovered of a rubber stamp impression made on a page positioned on top of the questioned document during shipping. The vivid image was surprising because it was not a concentrated pressure impression common to pen stroke indentations nor was it due to indentations from one page affecting the following page through friction.

Research duplicated the results. Several rubber stamp impressions using various stamps and inks were made on separate pages. Each stamped page was then placed over a blank page, using various paper types, and mailed across the country. Upon their return, the blank pages were processed with an electrostatic detection device. Several stamp images became clearly discernable. The transfers were subsequently determined to be caused by the inks' chemical components. The results further established images obtained from an electrostatic detection device are not restricted to common paper fiber disturbances associated with the pen pressure of writing.

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### **Indented Writing, Rubber Stamps, Document Examination**

## J23 Dating Watermarks

*Jane A. Lewis, MFS\*, Milwaukee, WI*

**Learning Overview:** After attending this presentation, attendees will develop an understanding of how to effectively visualize and decode the dates of watermarks on documents. Forensic Document Examiners (FDEs) will be shown practical methods to find paper industry contacts who can provide valuable watermark dating information for use in casework.<sup>1-4</sup>

**Impact Statement:** This presentation will impact the forensic science community by focusing awareness on how papers used in everyday business could contain watermarks. These watermarks may contain date codes that can be deciphered by FDEs with the aid of paper industry technical support.

The work of FDEs includes a variety of paper documents such as wills, trusts, quit claim deeds, personal guarantees, employment agreements, and real estate documents that may be genuine or fraudulent. If these questioned paper documents contain watermarks, the FDE must attempt to decipher the manufacturer's date of fabrication code, if one is incorporated. Watermarks are created at the wet end of the papermaking process by a dandy roll that typically contains wires on a cylinder with designs or printing to move the wet paper fibers away to create a watermark on the paper at the end of the process. Watermarks are visualized with transmitted light or simply holding the paper up to a bright light. Paper makers include watermarks in good-quality papers for the beauty of the designs and, in many instances, to date the paper. Watermark examples with date codes will be displayed.

Common paper industry directories of paper mills, paper manufacturers, and dandy roll makers from years ago have disappeared or changed to online formats. Paper companies and dandy roll makers have changed and consolidated over the years. Access to the latest paper industry directories for FDEs who occasionally need paper manufacturer information in casework can be difficult. The available directories come at a hefty price tag more suited for a large paper company to pay than a forensic scientist.

In two recent cases, watermarks were discovered on a questioned will, trust, and an agreement. The watermarks were visualized with transmitted light from a light box and a video spectral comparator. Two of the watermarks contained date codes and one did not. Watermark images and date codes will be illustrated. An internet search of the watermark company names revealed the paper manufacturers. Contact with a dandy roll maker in one case provided the date-coded year of manufacture of one watermark image. In another case, the papermaker's technical group provided the dating information after receiving an image of the watermark. Paper laboratories in Wisconsin and Georgia are good sources of information about paper manufacturers and dandy roll makers in the United States. Date coding information from the paper industry is proprietary and may be shared at their discretion. Approaching the paper technical workers in a professional way can produce their completely optional cooperation. Dating watermarks in casework is possible with the assistance of paper and dandy roll manufacturers.

### Reference(s):

1. SWGDOC Standard for Non-Destructive Examination of Paper, ver. 2013-1 accessed SWGDOC.org 8/29/2021.
2. Brunelle, R. and R. Reed. 1984. Forensic Examination of Ink and Paper. Charles C. Thomas Springfield, IL, pp. 211-221, 225-226.
3. Browning, B. 1977. Analysis of Paper, Second Edition, Marcel Dekker, Inc., New York, New York, pp. 332-333.
4. Scientific Examination of Questioned Documents, Second Edition. Edited by Jan Seaman Kelly and Brian Lindblom, 2006, CRC Press Boca Raton, Florida, pp. 297-301,347-34.
5. Hunter, Dard, 1947, Papermaking: The History and Technique of an Ancient Craft, Dover Publications, New York, NY pp. 258-308 and pp. 400-423

### Watermark, Forensic Document Examination, Paper

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## J24 Digital Image Processing for Determining Edge and Feature Detection for Pattern Recognition on the Shroud of Turin

Janis Winchester, MS\*, Fort Myers, FL

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of pattern recognition of possible symbols or letters given low-contrast image enhancement.

**Impact Statement:** This presentation will impact the forensic science community by providing information regarding determining edge characteristics of the visual impression on a document.

As an overview, the field of pattern recognition provides insights useful in forensic examinations, where there is a growing emphasis on digital documents. For example, the visual identification of a letter form is characterized by certain features. This process may be applied to letter formation where there is low-contrast digital information as described in, “Which Parts Determine the Impression of the Font?”<sup>1</sup>

Some definitions are provided for the attendees:

Pattern recognition—can be either “supervised,” where previously known patterns can be found in a given data, or “unsupervised,” where entirely new patterns are discovered.<sup>2</sup>

Perception “may be subjective and our eye-brain system is very efficient in supplying the missing information in order to make sense out of any pattern we can see.”<sup>3</sup>

Pareidolia, as noted in the *Merriam-Webster Dictionary*, “is the tendency to perceive a specific, often meaningful image in a random or ambiguous visual pattern.”<sup>4</sup>

The goal of this research is to evaluate digital imaging that provides the greatest information for pattern recognition on documents, such as the Shroud of Turin.

### Reference(s):

1. Masaya Ueda, Akisato Kimura, and Seiichi Uchida. Which Parts Determine the Impression of the Font? 2021 International Conference on Document Analysis and Recognition (ICDAR). Lausanne, Switzerland.
2. The Institute of Electrical and Electronic Engineers (IEEE). <https://www.techopedia.com/definition/4575/institute-of-electrical-and-electronics-engineers-ieee#what-does-institute-of-electrical-and-electronics-engineers-ieee-mean>. Accessed 17 June 2021 2:24 PM.
3. Pareidolia definition. *Merriam-Webster Dictionary*. <https://www.merriam-webster.com/dictionary/pareidolia>. Accessed 5 July 2021.
4. Paolo Di Lazzaro, Daniele Murra, and Barrie Schwartz. Pattern recognition after image processing of low-contrast images, the case of the Shroud of Turin. *Pattern Recognition* vol. 46, pp. 1964-1970 (2013).

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### Pattern Recognition, Document Analysis, Visual Impression



## K1 Phenibut, You Say What? A Phenibut and 2-Methyl AP-237 Overdose in a Sober Living Facility

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**Learning Overview:** After attending this presentation, attendees will understand the history and methods of detection for phenibut and have an awareness of drug-related deaths in sober living facilities, despite multiple negative drug tests during admission.

**Impact Statement:** This presentation will impact the forensic science community by presenting a case of a drug-related death in a sober living facility as a result of phenibut and 2-methyl AP-237.

A 29-year-old man with a history of benzodiazepine and gabapentin abuse, chronic gastrointestinal issues, back pain, and depression was found unresponsive at a sober living facility by his roommate. While normally introverted, the week prior to death the decedent was really chatty. The evening prior, the decedent appeared drowsy, so the roommate helped him into bed. When the roommate awoke in the morning, he noticed bloody purge emanating from the decedent's nose. 9-1-1 was called, naloxone was administered without improvement, and death was subsequently pronounced on scene. The sober living supervisor noted that residents undergo weekly drug testing and the decedent tested negative two days prior to his death.

Autopsy revealed mild pulmonary edema (right lung, 700 grams; left lung, 575 grams), left ventricular hypertrophy (1.7cm), and multinodular goiter. In-house comprehensive toxicology examination of blood revealed 2-methyl AP-237. Medical evidence was tested to help guide further toxicological analysis. A white powder collected at the scene revealed 4-phenyl-2-pyrrolidinone, and pill fragments collected from the small intestine during autopsy revealed 4-phenyl-2-pyrrolidinone, menthol, and nicotine. 4-phenyl-2-pyrrolidinone is an acidic compound and was not detected on an in-house comprehensive panel of blood specimens, which does not include an extraction for acidic drugs. Further research revealed this is the cyclic product of phenibut ( $\beta$ -phenyl- $\gamma$ -aminobutyric acid) that is created at high temperatures.

Toxicology from NMS Labs and the Center for Forensic Science Research and Education laboratory confirmed the presence of phenibut (77,000ng/mL) and 2-methyl AP-237 (5,800ng/mL), respectively. 2-methyl AP-237 is classified as a novel synthetic opioid with central and peripheral analgesic activity.

Phenibut is a central nervous system depressant that is not available for clinical use in the United States or most European countries. It was developed in the Soviet Union in the 1960s to treat insomnia, alcohol withdrawal, and anxiety. It was reportedly valued for its high tranquilizing effects during Russian space flights and is currently marketed as a nutritional supplement and mood enhancer, as it provides a sense of euphoria, reduced anxiety, and hypnosis. Drug tolerance has been reported to develop within a couple weeks of its use. It is purchased online by most users and cannot be readily detected, as there are no blood or urine tests available for commercial diagnosis. The levels detected in the decedent's blood were 22 times that of an average plasma concentration detected after a single, 250mg dose and 2.6 times that of a comatose woman.<sup>1-3</sup>

The cause of death was certified as combined effects of 2-methyl AP-237 and phenibut toxicity and the manner of death was listed as accident.

This case highlights the importance of collecting paraphernalia and apparent drugs during scene investigation as well as emphasizing the impact of a thorough examination of the gastric and intestinal contents for potential pill fragments, which may aid in toxicologic analysis of blood specimens in unconventional drug-related deaths.

### Reference(s):

1. Downes, M.; Berling, I.; Mostafa, A.; et al. Acute behavioural disturbance associated with phenibut purchased via an internet supplier. *Clin. Tox.* 2015; 53:636-638.
2. Isoardi, K.; Kulawickrama, S.; Isbister, G. Severe phenibut poisoning: An adolescent case cluster. *Journal of Paediatrics and Child Health* (2019). doi:10.1111/jpc.14605. Accessed March 4, 2020.
3. Sankary, S.; Canino, P.; Jackson, J. Phenibut Overdose. *American Journal of Emergency Medicine*. <http://dx.doi.org/10.1016/j.ajem.2016.08.067>. Accessed March 4, 2020.

### Phenibut, Drug-Related Deaths, Sober Living Facility

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## K2 Fentanyl Analogs in Dried Plasma Spots (DPS): A Novel Technique

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**Learning Overview:** After attending this presentation, attendees will better understand the use of Dried Plasma Spots (DPS) for the detection of fentanyl analogs.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with a novel DPS technique for the detection of fentanyl analogs.

Blood and urine are common biological matrices for drug detection in forensic toxicology, but the disadvantages of both matrices may cause individuals to be unwilling to participate in drug sampling. An alternative matrix for drug detection that offers minimally invasive collection and small sample sizes must be explored. DPS offer many advantages over common biological matrices and have potential to become suitable matrices for drug detection in forensic settings. There are limited studies that assess drugs of abuse in DPS. This study aimed to extract 13 fentanyl analogs from DPS.

The analytes of interest were methoxyacetylfentanyl, acetylfentanyl, acrylfentanyl, 4-ANPP, fentanyl, furanylfentanyl, para-fluorofentanyl, cyclopropylfentanyl, cis-3-methylfentanyl, carfentanil, butyrylfentanyl, 4-FIBF, and valerylfentanyl. A solvent extraction was optimized to isolate the analytes from Noviplex™ plasma preparation cards. This method was chosen to eliminate equipment cost and lengthy sample preparation. The assessed parameters of the solvent extraction included solvent type, solvent volume, internal standard placement, and blood sample volume. Neat standards and non-matrix controls were also evaluated in order to assess drug movement through DPS layers onto the plasma disc and to determine if whole blood hinders drug transfer compared to blood plasma samples. Briefly, whole blood was fortified with drug standards and 50µL was added onto the Noviplex™ cards. The blood spots were allowed to dry for 3min before the top layers were peeled away and discarded. The plasma discs were air dried for ten minutes before inserting into microcentrifuge tubes with deuterated internal standard and extraction solvent. Tubes were vortexed, sonicated, and dried under nitrogen. Samples were reconstituted in 25µL mobile phase and analyzed using a previously developed method on an Agilent® Technologies 1290 Infinity liquid chromatograph coupled to an Agilent® Technologies 6530 Accurate Mass Time-of-Flight (LC/qTOF) mass spectrometer operated in targeted acquisition mode.

Samples were best extracted using 1mL of 1% formic acid in a 50:50 methanol-acetonitrile solution. The extraction method was partially validated according to Academy Standards Board (ASB) forensic toxicology validation guidelines. The calibration ranges were 1–100ng/mL for all analytes. The limit of detection and lower limit of quantification for all analytes were 1 and 5ng/mL, respectively. Most analytes displayed unacceptable bias and precision at medium (40ng/mL) and high-quality (80ng/mL) control levels. In addition, large matrix effects were observed and extraction recoveries ranged from 5–7%.

This research introduces a novel technique for extracting fentanyl analogs from DPS using a simple extraction technique and robust instrumentation. While additional extraction optimization should be explored to increase recovery, analytical sensitivity may be suitable for overdose cases. DPS present the ability to serve as an alternative, novel matrix for future forensic toxicology applications.

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### Fentanyl Analogs, LC/qTOF, Dried Plasma Spots (DPS)

### **K3 Simultaneous Determination and Quantitation of Five Synthetic Cathinones in Postmortem Blood and Urine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

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**Learning Overview:** Concerned with frequent reports on abuse and deaths pertaining to overdose of synthetic cathinones, an effective LC/MS/MS method was developed for the analysis of five common synthetic cathinones (N-ethylpentylone, dibutylone, mephedrone, MDPV, and methedrone) in postmortem specimens to help determine victims' causes of death.

**Impact Statement:** Effective detection and identification of NPS in the laboratory facilitates data collection helpful to the formation of supply reduction and health intervention strategies.

**Methods:** For method evaluation, 1mL drug-free blood (or urine samples), fortified with 50–1,000ng/mL of the five analytes of interest along with respective deuterated internal standards, were used for the liquid-liquid extraction process, first with the addition of 2mL 1.5 M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> (pH 9.5) buffer for alkalization. Extraction was performed with 3mL dichloromethane:1,2-dichloroethane:*n*-heptane:ethyl acetate mixture (1:1:1:1, v/v). The extract was evaporated and reconstituted, then injected into the LC/MS/MS system. Chromatographic separation was performed at 50°C using an Agilent® ZORBAX® SB-Aq column (100mm × 2.1mm, 1.8µm particle). The mobile phase was consisted of water (0.1% formic acid) and methanol (0.1% formic acid) at a flow rate of 0.28mL/min. Mass spectrometric analysis was performed using electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode. The same protocol was applied to the analysis of case specimens.

**Results:** The protocol described above was evaluated and found to achieve the following analytical parameters for blood samples: (1) average extraction recovery range: 73.3–108.5%; (2) average matrix effect ranges: 90.3–149.0%; (3) intraday and interday precision ranges (percent CV):0.95–12.6% and 1.46–10.9%; (4) intraday and interday accuracy ranges: 96.7–105.9% and 98.2–103.2%; and 5e) calibration linearity (r<sup>2</sup>), detection limit, and quantitation limit: >0.999, 0.2–0.5ng/mL, and 0.5ng/mL, respectively. The corresponding parameters achieved for urine samples were as follows: (1) 74.5–91.8%; (2) 82.5–113.6%; (3) 0.56–9.81% and 1.35–11.3%; (4) 88.6–105.3% and 95.2–102.6%; and (5) >0.999, 0.5ng/mL, and 0.5ng/mL, respectively. This protocol was found effective for the analysis of these synthetic cathinones in postmortem samples. In 2020, blood specimens from cases were found to contain at least one of these five synthetic cathinones with the following mean concentrations (µg/mL) and number of cases: N-ethylpentylone (0.46; n=6); dibutylone (0.36; n=8); mephedrone (0.73; n=57).

**Conclusions:** Synthetic cathinones played a significant role in fatalities and were often abused simultaneously with ketamine, PMMA, and sedative drugs in Taiwan. With no or limited understanding on these substances' toxicity, addiction potential, and withdrawal symptoms, growing use of New Psychoactive Substances (NPS) is certainly a critical public health issue. Effective detection and identification of NPS in the laboratory facilitates data collection, helpful to the formation of supply reduction and health intervention strategies.

#### **New Psychoactive Substances, Synthetic Cathinones, Postmortem Toxicology**

## K4 Trazodone-Related Deaths in Taiwan From 2011 to 2020: A Report on 591 Fatalities

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**Learning Overview:** Trazodone is an antidepressant medication used for the treatment of major depressive disorder, anxiety disorder, and alcohol dependence. Since trazodone is often present in postmortem specimens from accidental, suicidal, and homicidal poisoning cases, an effective Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed and applied to the analysis of this compound in postmortem samples to aid in determining the causes of death.

**Impact Statement:** These epidemiological data reveal important information related to the circumstances and usage of trazodone in Taiwan.

**Methods:** Postmortem blood specimens were routinely screened for trazodone using liquid-liquid extraction (by a Toxi-tubes® A protocol), followed by Gas Chromatography/Mass Spectrometry (GC/MS), LC-ion-trap/MS, and Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) methodologies (including an automated library search step) developed earlier in the laboratory. Positive specimens were confirmed for trazodone and quantified by LC/MS/MS using trazodone-d<sub>6</sub> as the internal standard. This method has been validated and found to achieve 0.005µg/mL as the limit of quantitation. Complete autopsy records for these 591 cases were reviewed and analyzed against analytical findings to reveal key epidemiological data.

**Results:** Among the 32,140 forensic autopsy cases that took place during the period (2011–2020) included in this retrospective study, specimens collected from 591 cases were found to contain trazodone. Yearly distributions of these trazodone-positive cases were: 2020, 33; 2019, 38; 2018, 34; 2017, 60; 2016, 92; 2015, 69; 2014, 81; 2013, 54; 2012, 67; and 2011, 63. Among these 591 fatalities, the mean age was 49.0 (ranging from 21 to 91) and 61.4% were male ( $n=363$ ). The manners of death for these cases were 46.5% accident ( $n=275$ ), 24.2% suicide ( $n=143$ ), 18.1% natural death ( $n=107$ ), 7.3% uncertainty ( $n=43$ ), and 3.9% homicide ( $n=23$ ); while the causes of death were 38.2% intoxication—including single and multiple drugs ( $n=226$ ), 26.6% hypovolemic and neurogenic shock ( $n=157$ ), 21.7% drowning or other asphyxia ( $n=128$ ), 11.0% cardiogenic shock ( $n=65$ ), and 2.5% charcoal-burning suicides or fire disaster ( $n=15$ ). Blood trazodone concentrations ranged from 0.01 to 97.0µg/mL; mean concentrations (with respect to manner of death) were 0.94, 3.21, 0.28, 1.38, and 1.24µg/mL for accident, suicide, natural death, uncertainty, and homicide cases, respectively; mean concentrations (with respect to cause of death) were 2.56, 0.30, 1.07, 0.45, and 2.44µg/mL for intoxication, hypovolemic and neurogenic shock, drowning or other asphyxia, cardiogenic shock, and charcoal-burning suicides or fire disaster, respectively. Alcohol, sedative drugs, and antidepressant drugs were the most common companion compounds presented in trazodone-related cases. Alcohol was detected in 208 cases (35.2%) and the numbers of cases found to contain other drugs were: 149 for flunitrazepam (25.2%); 147 for clonazepam (24.9%); 132 for morphine (22.3%); 122 for codeine (20.6%); 99 for estazolam (16.8%); 96 for zolpidem (16.2%); 87 for quetiapine (14.7%); 71 for acetaminophen (12.0%); and 65 for methamphetamine (11.0%).

**Conclusion:** Trazodone was commonly found in blood specimens collected from Taiwanese forensic autopsy cases; 61.4% of these 591 fatalities occurred during the 2011–2020 period were male, while the mean age was 49.0. Among these trazodone-positive cases, the most common manner of death and cause of death were accident (46.5%) and intoxication (38.2%), respectively. These epidemiological data reveal important information related to the circumstances and usage of trazodone in Taiwan.

### Trazodone, Postmortem Toxicology, Epidemiology

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## K5 A Study on the Characteristics of New Psychoactive Substances (NPS) in Korea From 2014 to Recent Years

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**Learning Overview:** After attending this presentation, attendees will understand the recent status of NPS in Korea and the detection of synthetic cannabinoids in the hair of drug users.

**Impact Statement:** This presentation will provide the forensic community with the NPS trends in Korea and data from the detection of synthetic cannabinoids in the hair of drug users from 2014 to recent years.

In 2020, the number of drug offenders in Korea was about 18,000, which was double the number compared to 2010. In this regard, the number of drug-related cases in the National Forensic Service (NFS) was about 68,000 in 2020, a 2.4-fold increase compared to 2014. The first appearance of NPS in Korea was in 2009, and the number of NPS detected in cases at that time was only 8; however, the number was 184 in 2019, a 24-fold increase compared to a decade ago. The government introduced the “Temporary Narcotics Designation System” in 2011 and began to preemptively regulate NPS. Despite all that, the simple reconstruction (producing process) of NPS allows for a large number of NPS that evade the drug law. In this study, the status of NPS abuse in Korea was investigated, focusing on NPS that were detected in hair of drug users from 2014 to recent years.

As of June 2021, a simultaneous analytical method was established using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for 25 synthetic cannabinoids and 27 of their metabolites in hair samples. From 2014 to May 2021, a total of 19 synthetic cannabinoids were detected in the hair of drug users. Annual detection cases ranged from 4–64 cases, and the highest was in 2020 with 64 cases. Among the detected NPS, AB-CHMINACA was the most detected with 43 cases, followed by 4F-MDMB-BUTINACA (32 cases), 5F-ADF (27 cases), JWH-210 (20 cases), 5F-MDMB-PICA (20 cases), AMB-FUBINACA (16 cases), and MDMB-4en-PINACA (14 cases). In particular, MDMB-4en-PINACA first appeared in Korea in 2021 and is estimated to be a major NPS of abuse in the future. It was found that one type of NPS used to be solely used; however, unlike in the past, it is now common that two or more NPS are being used in a mixed form. In addition, it used to take at least a year for another new drug to come to market, but now NPS gain popularity more quickly and change more frequently.

In order to prove the use of NPS, it is important to develop an analytical method from biological samples, which is usually carried out in four steps: information collection, Reference Standard Material (RSM) and metabolites obtainment, method development and validation, and application to authentic samples. It takes a long time to obtain RSM, making it difficult to develop analytical methods. Considering the characteristics of NPS being used at an exceptionally fast rate, the government needs to introduce a fast track system to shorten the time required to secure RSM and provide budgets to secure research personnel and high-resolution equipment.

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### New Psychoactive Substances (NPS), MDMB-4en-PINACA, Fast Track System

## K6 Death Via Ingestion of Zinc Phosphide

Erin Spargo, PhD\*, NMS Labs, Horsham, PA; Beth Slaybaugh, MPS, NMS Labs, Horsham, PA; Lindsay Cheeseman, BS, NMS Labs, Horsham, PA; Lee Blum, PhD, NMS Labs, Horsham, PA

**Learning Overview:** After attending this presentation, attendees will be familiar with the effects of zinc phosphide poisoning in the context of a presented case report, including autopsy and toxicological findings.

**Impact Statement:** This presentation will impact the forensic community as attendees will be better prepared to interpret postmortem findings from zinc phosphide poisonings.

A Be On the Lookout (BOLO) was issued for a 28-year-old White male after he made suicidal statements to local law enforcement. Employed as an exterminator, he was found deceased in the driver's seat of his work truck. His statements indicated that he had consumed gopher poison. Multiple containers of "Mole and Gopher Bait," containing zinc phosphide, were found near the body.

An autopsy revealed both internal and external decompositional changes. Except for the heart, examination of the organs and tissues was performed *in situ*. Congested, collapsed parenchyma were observed in the lungs. Various biological specimens were collected; the medical examiner did not collect gastric contents due to the potential toxicity of the sample. Liver, femoral blood, vitreous fluid, and urine were submitted to the laboratory.

Liver homogenate was screened via Enzyme-Linked Immuno-Sorbent Assay (ELISA) for 12 drugs/drug classes and headspace gas chromatography was used to screen for alcohols. Confirmation testing for cannabinoids was performed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Blood was analyzed for zinc via Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). The sample was diluted with a solution containing the internal standard, then introduced into the ICP/MS for analysis.

The results of the testing showed the liver homogenate was positive for delta-9-THC with 1,800ng/g delta-9-carboxy THC. Zinc was identified in the blood at a concentration of 1,400µg/dL (14mg/L); for reference, in one study, postmortem blood zinc concentrations in non-poisoned individuals ranged from 4.0–8.7mg/L.<sup>1</sup>

Zinc phosphate exhibits its toxicity by the creation of phosphine gas when the material interacts with the acidic, aqueous gastric fluid. The gas is rapidly absorbed, interfering with the respiratory cycle of cells, causing oxidative tissue damage. Symptoms observed after ingestion can be multiphase. Initially, toxicity manifests primarily as gastrointestinal discomfort, with symptoms including nausea, vomiting, and diarrhea. This can be followed by a symptom-free period. Ultimately, multi-organ failure occurs, with cardiopulmonary injury the primary cause of early death. There is no known antidote, resulting in a high mortality rate. Limited toxicological data are available regarding deaths due to zinc phosphide ingestion. Case reports are not generally supported by toxicological findings and often rely on a case history of ingestion.

The medical examiner ruled the cause of death as zinc phosphide ingestion, supported by the presence of the poison near the body, notes of suicidal intent, congested lungs, and an elevated concentration of zinc in the blood.

### Reference(s):

<sup>1</sup>. Lech, T.; Sadlik, J.K. Zinc in postmortem body tissues and fluids. *Biological trace element research*. 2011; 142(1): 11-17.

### Forensic Toxicology, Zinc Phosphide, Postmortem

## K7 Profiling Trace Element Contaminants of Toxicological Interest in Commercially Available Cannabidiol (CBD) Tincture Oils

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**Learning Overview:** Attendees of this presentation will learn: (1) the prevalence of heavy metal contamination for the currently regulated metals (Mercury [Hg], Lead [Pb], Cadmium [Cd], and Arsenic [As]) in commercial CBD oils, (2) whether sufficient evidence exists to support expansion of the list of regulated metals, and (3) the effectiveness of current attempts at industry self-regulation.

**Impact Statement:** Heavy metals contamination/poisoning is often overlooked given the chronic nature of its affects. This presentation will highlight concerns regarding the potential for heavy metals poisonings as identified through a “market basket” study of readily available CBD consumer products.

**Background:** There is legitimate concern over the rise in chronic health issues related to metals contamination in CBD-infused products. Hemp, a hyperaccumulator plant, boasts the high biomass, long roots, short life cycle, and genetic structure ideally suited for absorbing and accumulating heavy metals. The situation is exacerbated by the popularity of these products. Sales to United States consumers in the form of pharmaceuticals, dietary supplements, foods, and other consumables are expected to grow >20% annually and reach ~\$25 billion dollars by 2025. As demand outpaces supply, hemp plants are being sourced from outside of the United States and, in some cases, from countries having questionable environmental policies. Yet despite these facts, the regulatory environment is highly fragmented with inconsistent product standards and testing protocols from one state to another.

**Objectives:** The objective of this study was to document the trace metal profile in a sampling of commercially available full spectrum CBD tincture oils with the aim of:

- identifying and quantifying metal contaminants of toxicological interest
- assessing truth in labeling relative to those metals that are regulated
- assessing the adequacy of the scope of state-mandated heavy metals testing requirements

**Methods:** A “market basket” study of 18 hemp oils was conducted. The samples were full-spectrum CBD oils having a potency of 25–50mg/mL CBD and naturally flavored (although this was not possible with all producers). Variables that could not be controlled given the guidelines presented include method of solvent extraction (supercritical fluid vs. ethanol extraction), final solvent (Medium Chain Triglycerides [MCT], olive oil, or hemp oil), and the source of the hemp from which the CBD was derived.

Samples were prepared using microwave digestion with each batch, including a digestion blank and Quality Control (QC) standard followed by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) analysis for > 40 elements. Three replicate digests of each sample were analyzed. Three different lots of material were purchased so that temporal (i.e., lot-to-lot variation) could be studied.

**Results:** The majority of elements monitored, including Hg, As, and Cd, were either below the limit of quantification or below regulated levels. Only the samples diluted in hemp oil were found to contain levels of Manganese (Mn) as high as 0.73 +/- 0.06ppm. For reference, the Food and Drug Administration (FDA) has established that the Mn concentration in bottled drinking water should not exceed 0.05ppm, and the World Health Organization (WHO) drinking water quality guidelines for Mn are 0.4ppm. At least two samples were found to contain elevated levels of lead, 1.59 +/- 0.14ppm and 3.64 +/- 0.08ppm, nearly 3 to 7 times state-regulated levels of lead in cannabis products. The published certificate of analysis for the sample containing 1.59ppm of lead indicated that the lead concentration was below the regulated level at the time the product was tested. The published certificate of analysis for the sample containing 3.62ppm of lead did not indicate that any metals testing was performed on the final product.

**Conclusion/Discussion:** The potential exists for heavy metal contamination in consumer products, despite current regulations that can be ambiguous and fragmented. A question exists as to whether the elevated lead levels identified are the result of the biomass, contamination of the product by the packaging material, or poor testing protocols. Additional assessments regarding lot-to-lot reproducibility and any identified correlations between geography, potency, CBD extraction method, etc. will be presented.

### Cannabidiol, CBD, Metals

## K8 A Toxicological Analysis of Methadone and Oxycodone in Decomposition Fluid and Liver Tissue From Decomposed Bodies

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**Learning Overview:** After this presentation, attendees will have gained an understanding of the limitations of postmortem toxicological analysis and will have learned about a possible new method for postmortem drug testing.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence that decomposition fluid may serve as a suitable specimen for postmortem toxicological analysis.

The Centers for Disease Control and Prevention report that over the past 20 years, nearly 841,000 people have died from a drug overdose in the United States. In 2019 alone, there were 70,630 deaths attributed to a drug overdose.<sup>1</sup> Deaths from drug overdose represent an enormous public health burden and continue to contribute to the increasing caseloads of forensic pathologists. As a result, postmortem toxicological analysis is an essential part of the autopsy investigation and is required for an accurate determination of cause of death.

Postmortem toxicological analysis is most commonly performed on blood, liver, and urine specimens.<sup>2</sup> In the case of decomposed bodies, though, the availability of these preferred specimens for analysis is often limited. Ketola and Kriikku recommend using skeletal muscle for postmortem toxicological analysis when blood is not available.<sup>3</sup> However, it is difficult to produce a homogeneous specimen for toxicological analysis from muscle tissue. As a result, drug extraction can be arduous and accurate determination of extraction efficiency cannot be made with certainty. In addition, there is substantial sample site variability in drug concentrations derived from muscle tissue.<sup>4</sup> Lastly, while there is considerable literature on the quantitative analysis of drugs in liver tissue, there is limited information on the interpretation of quantitative drug analysis in muscle tissue.

Despite the challenge of toxicological analysis of limited specimens from decomposed bodies, the need for testing remains. Thus, novel approaches for overcoming this challenge must be developed. This study sought to determine whether decomposition fluid, an often-abundant and easily acquired specimen in decomposed remains, could be utilized for postmortem toxicological analysis. Specifically, this study asked whether methadone and oxycodone could be qualitatively and quantitatively detected in both liver tissue and decomposition fluid from decomposed autopsy specimens.

This study assessed drug levels in decomposition fluid and liver tissue from decomposed remains over a 15-year period from a total of 680 autopsy cases. Drug levels were assessed using an alkaline liquid-liquid extraction with quantitation by gas chromatography with nitrogen phosphorus detection. Confirmation of drug levels was performed using gas chromatography/mass spectrometry. In 103 autopsy cases, methadone was detected in both decomposition fluid and liver tissue, with an average ratio of distribution of 1:12.7 (decomposition fluid: liver) and a standard deviation of 16.3. In 29 autopsy cases, oxycodone was detected in both decomposition fluid and liver tissue, with an average ratio of distribution of 1:1.5 (decomposition fluid: liver) and a standard deviation of 2.4. There were no cases where methadone or oxycodone were detected alone in decomposition fluid or liver tissue. These results demonstrate that methadone and oxycodone can both be detected in decomposition fluid and liver tissue and that decomposition fluid is a suitable specimen for postmortem toxicological analysis from decomposed autopsy remains. Further studies are ongoing to assess the applicability of decomposition fluid toxicological analysis for additional drugs and metabolites.

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### Toxicology, Decomposition, Liver



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## K9 The Effects of Ethanol on GABA Catabolism: Succinyl Semialdehyde Dehydrogenase

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the metabolism of the inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (Gamma-Aminobutyric Acid [GABA]), and the effect of ethanol on GABA catabolism.

**Impact Statement:** This presentation will impact the forensic science community by presenting a possible mechanistic explanation for some aspects of the GABA-nergic Central Nervous System (CNS) depressant effect of ethanol. This knowledge may help forensic toxicologists and pathologists understand the toxic response of ethanol in Drug-Facilitated Sexual Assault (DFSA), postmortem, and impaired driving cases.

Currently, ethanol is one of the most popular drugs of abuse, more so than either tobacco or marijuana. Ethanol is commonly encountered in many forensic toxicology cases, including DFSA, postmortem, and impaired driving cases. Ethanol can exacerbate the toxicity of other drugs that may be present by causing a greater depressant effect in the CNS. Despite its popularity, regularity, and importance in forensic toxicology casework, the mechanism of action for ethanol is not completely understood.

In the CNS, GABA is the primary inhibitory neurotransmitter and acts as a CNS depressant. When released by inhibitory neurons, GABA acts as an agonist of two GABA receptor types (GABAA and GABAB; activating chloride and potassium channels, respectively). GABA-Transaminase (GABA-T) and Succinyl Semialdehyde Dehydrogenase (SSADH) represent the first two enzymatic steps associated with the catabolism of GABA.

This research is directed toward the function of ethanol as a possible competitive inhibitor of SSADH. Because of the structural homology between the C1 and C2 carbons of ethanol and the C3 and C4 carbons of GABA, it is reasonable to conclude that ethanol could occupy the enzymatic active site of both SSADH and GABA-T. Such inhibition would be expected to increase the half-life of the substrate GABA, exacerbating CNS inhibition contributing to the characteristic CNS depression associated with ethanol consumption.

A commercially available enzymatic assay was adapted for evaluation of ethanol-based inhibition of GABA metabolism via a series of substrate-velocity experiments. This assay allowed for the determination of kinetic parameters using  $K_m$  (Michaelis-Menten coefficient) and  $V_{max}$  (maximal reaction rate). Initial experiments indicated that competitive inhibition was present at physiologically relevant concentrations (e.g.  $<0.2\text{g/dL}$ ) and is concentration dependent. Maximal velocity of the enzyme assay was unaffected by the addition of various concentrations of ethanol, and enzyme affinity for GABA decreased, with a corresponding increase in  $K_m$ . The inhibitory effect of ethanol on SSADH suggests a potential role for this process as a component mechanism of the CNS depressant effect of the drug.

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### GABA, Ethanol, Enzyme Kinetics

## K10 The Effects of Storage Time, Adverse Storage Conditions, and Lab Exposure on Blood Alcohol Concentrations

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**Learning Overview:** After attending this presentation, attendees will have learned about the effects of storage time, adverse storage conditions, and laboratory exposure on the Blood Alcohol Concentration (BAC) of blood tubes containing sodium fluoride as a preservative.

**Impact Statement:** This presentation will impact the forensic science community by providing further support to counter the common defense challenge of ethanol production in blood tubes due to storage time, storage conditions, or exposure to microorganisms in the laboratory.

In 1973, Blume and Lakatua reported the production of ethanol in a blood tube that contained 1% sodium fluoride.<sup>1</sup> In 1989, Chang and Kollman also reported this issue.<sup>2</sup> Defense lawyers often cite these publications when challenging the findings of a blood alcohol analyst in court, arguing the result is a falsely elevated BAC. Since these publications, multiple studies have investigated this issue; many of these studies under acceptable laboratory conditions, such as refrigerating blood tubes. This study focused on whether ethanol production could occur when conditions are not ideal. This study was conducted in the Houston Laboratory of the Texas Department of Public Safety. All blood tubes contained 1% sodium fluoride with BACs varying from 0.000 to 0.3,217g/100mL, which originated from a proficiency test provider. A Shimadzu® Gas Chromatography/Flame Ionization Detector (GC/FID) was used for the analysis.

The first part of this study examined storage time. Blood tubes stored between 2014 and 2021 in a refrigerator were each analyzed for the current BAC in July 2021. The BACs from July 2021 were compared to the original BACs reported when the tubes were first analyzed in the laboratory. No ethanol production occurred during the time the tubes were stored, which encompassed a range of months to years.

The second part of this study examined temperature and was conducted while the laboratory was undergoing air conditioning repair. As such, temperatures in the laboratory rose above the normal room temperature. Fifty percent of the blood tubes were left on the benchtop while the other 50% were stored in the refrigerator. The BACs from this part of the study were compared to the BACs collected from the first part of the study. No ethanol production occurred after more than ten days of storage under either storage condition.

The third part of this study examined whether areas of the laboratory contained microorganisms that, once in the blood tube, could produce ethanol. The samples used for this portion of the study were the previously refrigerated blood tubes. Blood tubes were exposed to swabs from various areas of the laboratory. One pair of blood tubes were allowed to remain open next to each other in the biohazard cabinet for ten minutes. Two replicates of each sample were made: one was left on the benchtop and the other was stored in the refrigerator. The BACs from this part of the study were compared to the BACs after the second part of the study. No ethanol production was detected after a week of storage.

Significant ethanol loss was only detected for samples from the first and third parts of the study. These results support the hypothesis that unusual temperatures and adverse laboratory exposures that may occur are unlikely to result in any ethanol production.

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### Ethanol, Blood Alcohol Concentration, Storage Conditions

## K11 *In Vitro* Metabolic Profile of the New Synthetic Opioid Bucinnazine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will understand the main phase I and phase II metabolites of bucinnazine, a new synthetic opioid.

**Impact Statement:** This presentation will impact the forensic science community by describing the metabolic processes of a new synthetic opioid.

Bucinnazine, also known as AP-237, is a synthetic opioid recently discovered in seized heroin samples in the United States and in Europe.<sup>1</sup> It was first synthesized in the late 1960s and has been used for the treatment of cancer-associated chronic pain in China for many years.<sup>2</sup> Bucinnazine is one of the most potent compounds among the series of piperazines, which also include other relevant compounds, such as MT-45, AD-1211, and 2-methyl-AP-237, a methylated derivative of bucinnazine. Bucinnazine is considered a  $\mu$ -selective opioid, binding primarily to the  $\mu$ -opioid receptor.<sup>3</sup> However, bucinnazine also may share several characteristics with other piperazines, which act primarily on dopamine, serotonin, and norepinephrine neurotransmission.<sup>4</sup> At present, bucinnazine is not scheduled in the United States, as it is not a therapeutic choice for the treatment of pain. Nevertheless, with the advent of the crypto currency and the easy access of substances on the Darknet, bucinnazine is a real threat to the public health.<sup>5</sup>

The metabolic profile is an important tool for the development of analytical methods for the screening of emerging synthetic opioids, as the metabolites act as biomarkers of previous use. Little information describes the bucinnazine metabolites, with *p*-hydroxylation being the main metabolic pathway.<sup>6</sup> Despite previous studies, little research has been completed on bucinnazine metabolism.

The aim of this study was to identify the *in vitro* phase I/II metabolites of bucinnazine by using pooled rat liver microsomes. Bucinnazine hydrochloride was incubated at 37°C for one hour with Sprague-Dawley rat microsomes for phase I metabolites. For phase II, UDGPA was added to an aliquot of the resultant phase I solution, and the mixture was incubated at the same conditions for an additional one hour. Analyses were carried out using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and Direct Analysis in Real-Time-Time-Of-Flight/Mass Spectrometry (DART<sup>®</sup>-TOF/MS). The DART<sup>®</sup>-TOF/MS analysis was performed for the untargeted screening of the metabolites' exact masses. For this analysis, a capillary tube was dipped in a methanolic solution of the obtained metabolites and introduced into the helium stream of the DART<sup>®</sup>-TOF/MS. For identification of the metabolites, LC/MS/MS analysis was performed. A volume of 2 $\mu$ L was injected onto a Shimadzu<sup>®</sup> LC/MS/MS 8050 system equipped with a Poroshell HPH C18 column with a gradient mobile phase consisting of 5mM ammonium formate and 0.1% formic acid in water as mobile phase A, and 100% methanol as mobile phase B. A flow rate of 0.500mL/min and a dwell time of 1ms was applied. Fentanyl-d5 was used as the internal standard. The ion transitions for bucinnazine and fentanyl-d5 were 273 *m/z*  $\rightarrow$  117 *m/z* and 273 *m/z*  $\rightarrow$  91 *m/z*, and 342*m/z*  $\rightarrow$  105 *m/z* and 342 *m/z*  $\rightarrow$  188 *m/z*, respectively.

Both phase I and phase II metabolites, including the monohydroxy metabolites, were detected. This confirms *p*-hydroxylation as the main metabolic pathway of bucinnazine. The method was successfully applied to urine samples of patients suspected of previous bucinnazine use. Given the increase in illicit bucinnazine cases in the United States, a deeper understanding of its metabolites as biomarkers is important for the development of reliable identification strategies to detect bucinnazine misuse cases.

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### Bucinnazine, AP-237, Metabolism

## **K12 The Validation of a Screening Method for 700+ New Psychoactive Substances (NPS) in Authentic Urine by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)**

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**Learning Overview:** After attending this presentation, attendees will understand the potential implications of a screening method including 700+ NPS in forensic toxicology.

**Impact Statement:** This presentation will impact the forensic community by providing a comprehensive screening method that is high throughput and reliable for the analysis of NPS in human urine.

In forensic toxicology, the most common techniques for screening of licit and illicit drugs are immunoassays and Gas Chromatography/Mass Spectrometry (GC/MS). These methods screen for compounds using specific target structures and chemical properties and are well established for routine drug analysis. However, NPS typically have structural modifications that can result in false positive and negative detections using these techniques. Newer techniques such as LC/QqQ/MS allow for less sample preparation, increased sensitivity, analysis of biological samples without derivatization, and separation of small, polar, and non-volatile compounds. Consequently, LC/QqQ/MS holds many advantages for screening of NPS.

This research reports the validation results for an LC/QqQ/MS dynamic Multiple Reaction Monitoring (dMRM) method for 713 NPS and other drugs in urine using a dilute-and-shoot approach. The method was then applied for screening of several hundred authentic urine samples obtained from a drug testing laboratory. Method validation was completed using reference standards, including deuterated standards, obtained from Cayman Chemical as a neat solid or as a solution. Sixteen mixtures, each containing 25–70 analytes, were created based on the structure of the compound, retention time, drug class, and transitions that were previously collected by LC/QqQ/MS and incorporated into an in-house database (Agilent® PCDL). Mixtures were designed to minimize co-elutions and reduce interferences for reliable identification and analysis of the compounds. Validation parameters were based on the Organization of Scientific Committees (OSAC) recommendations and parameters that were chosen as most applicable for screening validation included bias, carryover, Limit Of Detection (LOD), and ion suppression/enhancement. LODs for all analytes ranged from 0.002 to 0.169ng/mL, which are below levels typically reported for NPS in human specimens.

The validated method was used to screen for NPS in authentic urine specimens that were collected in 2013–2014 and stored at -20°C. Urine samples were collected from individuals in addiction treatment and pain medication monitoring programs and were supplied de-identified. Samples were processed using a dilute-and-shoot method in which 100µL of sample was added to 500µL of High-Performance Liquid Chromatography (HPLC) water. Urine samples were analyzed with and without glucuronidase treatment. Numerous positive detections of NPS, including synthetic cathinones and cannabinoids, and other licit and illicit compounds, were determined in these specimens, indicating that the method may have value as a comprehensive NPS screen in forensic toxicological analysis.

### **Method Validation, New Psychoactive Substances, Forensic Toxicological Screening**

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**G13 Gender Differences in Driving Under the Influence (DUI) of Psychoactive Drugs: A Systematic Review and Meta-Analysis**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the prevalence of Driving Under the Influence (DUI) of psychoactive drugs in male and female drivers.

**Impact Statement:** This presentation will impact the forensic science community by presenting the first systematic review and meta-analysis on gender differences in DUI drivers.

As observed in epidemiological studies, and in particular in two recent studies, a higher percentage of drivers positive for drugs of abuse are normally found in males, while medicines are more frequently found in females.<sup>1-3</sup> However, scientific literature shows that studies are heterogeneous in sample size, inclusion criteria, or characteristics of the analyzed population, limiting the strength of the resulting evidence.

This study performed a broad systematic search of papers reporting toxicological results on selected matrices (blood, serum, plasma, saliva) showing gender differences.<sup>4</sup> Meta-analysis of random effects estimates was performed to investigate the change in the overall effect size (by Cohen d standardized mean difference test). Mann Whitney U test was performed to test for differences between gender. From the more than 5,000 records identified, after exclusion of duplicates and title/abstract screening, 613 papers were included for full-text analysis. Four hundred fifteen papers were excluded because they lacked original toxicological results, 131 because the gender differences were not considered, and 25 because only data on alcohol were reported.

A total of 27 papers were included, and the total population sample consisted in 100,485 drivers (70,110 men and 30,321 women). The meta-analysis showed a significant higher prevalence among men for cocaine (1.8% vs. 0.9%;  $p<0.001$ ), delta-9-tetrahydrocannabinol (3.5% vs. 1.6%;  $p<0.01$ ), and amphetamine-like drugs (1.2% vs. 0.6%;  $p<0.01$ ), as expected. Surprisingly, no differences were observed in the use of opiates (2.3% vs. 2.2%;  $p=0.45$ ) and benzodiazepines/Z-drugs (2.9% vs. 3.7%;  $p=0.52$ ). This systematic review and meta-analysis shows that, contrary to the extraordinary amount of epidemiological analysis performed on drivers, only a few studies differentiate the prevalence between male and female drivers, not allowing the comparison and analysis of aggregated data. This can lead to an underestimation of the influence of gender in DUI phenomenon or complicate the evaluation of the results as observed for medications, which did not show significant differences despite several studies indicating their higher prevalence in females. The primary goal in the future will be to collect data of DUI drivers following shared and homogeneous methodologies in order to allow for the analysis of larger aggregated samples, which can be used for targeted prevention and enforcement efforts, as well as monitoring evolving trends.

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**Forensic Toxicology, Driving Under the Influence, Gender**

## K14 A Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS) Determination of Ethyl Glucuronide (EtG) on Real Hair Samples and a Medicolegal Evaluation of Alcohol Use and Abuse

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**Learning Overview:** After attending this presentation, attendees will have an insight on an application of an innovative and solid GC/MS/MS method for the evaluation of EtG on medicolegal cases, which were provided to this study for research purposes.

**Impact Statement:** This presentation will impact the forensic science community by outlining a connection between toxicological analysis on conventional matrices and medicolegal evidence, using the analysis of EtG to provide further information about a subject's history in alcohol use and abuse.

EtG is a minor non-oxidative ethanol metabolite, resulting from the enzymatic glucuronidation of ethanol in the liver.<sup>1,2</sup> This small molecule can be detected in several body fluids and tissues for variable intervals of time after ethanol ingestion. Furthermore, there is a growing interest in its use in epidemiological studies in order to investigate drinking patterns and correlation to chronic disease and mortality. Currently, the consensus of the Society of Hair Testing (SoHT) describes that concentration of EtG in hair is expected to be above a cut-off value of 30pg/mg for chronic heavy drinkers, corresponding to a daily ethanol intake >60g.<sup>3</sup>

The evaluation of EtG on keratinic matrices to investigate the history of alcohol use and abuse in an individual is not usually carried out to assess the cause of death. It can, however, provide extremely valuable information for the investigation when the case is one of legal relevance.

For EtG analysis, the specimen was decontaminated by washing with organic solvents, then dried and finely shredded in 1–2mm fragments; 50mg of sample was then put overnight in 2mL of water with an appropriate concentration of internal standard EtG-d5. The supernatant was purified with cationic bed Solid-Phase Extraction (SPE) and derivatized with MSTFA before analysis in Gas Chromatography-Electron Ionization-Triple Quadrupole (GC-EI-QQQ).

In this study, female subjects whose toxicological analysis had been requested following autopsy to help determine cause of death were considered. On both subjects, a high value of alcohol had been found in conventional matrices such as central and peripheral blood, vitreous humor, urine, and gastric content. Therefore, it was interesting to analyze whether these values may be the result of a sporadic assumption of alcohol or coherent with an alcoholic behavior. For this reason, EtG analysis was carried out on hair samples, both on a full-length sample to determine an average concentration in the months before death and on multiple segments to evaluate the history of alcohol consumption over time.

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### Alcohol Abuse, EtG, Hair Samples

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## **K15 The Adulteration of Powder Milk (Lactogen-1) With Lambda-Cyhalothrin Causing the Death of a Baby Girl: A Case Study**

*Hafiz Faisal Usman, MS\*, Forensic Toxicology, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Muhammad Imran, MPhil, Forensic Toxicology, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Asif Raza, MPhil, Forensic Toxicology, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Muhammad Amjad, PhD, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan; Mohammad Ashraf Tahir, PhD, Punjab Forensic Science Agency, Lahore, Punjab, Pakistan*

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**Learning Overview:** After attending this presentation, attendees will have a better view of the forensic toxicologist's contribution in solving cases with unknown causes of death.

**Impact Statement:** This presentation will provide a guide for the analysis of the most challenging exhumation specimens. Forensic toxicologists regularly analyze blood, urine, and tissue specimens, but exhumed specimens with varying degrees of putrefaction pose a greater analytical challenge.

**Case History:** A one-month-old baby girl had severe shortness of breath and seizures after consuming a Nestlé® product (lactogen-1) and was taken to a hospital. However, she did not survive. Cardiopulmonary arrest was declared as a cause of death. After religious rituals, she was buried, but her father questioned the quality of powder milk. Due to the matter of suspicion, police collected the packet of remaining powder milk and a water bottle used for dilution of milk. A portion of the milk was sent to Nestlé® for quality testing. Nestlé's® laboratory testing indicated the presence of excessive amounts of calcium chloride, sodium, and potassium in the milk. The victim's father requested through the court of law an exhumation and postmortem of his child. After 75 days of burial, the victim's body was exhumed. The body was in an advanced stage of putrefaction due to the hot and humid weather in Pakistan. The abdominal cavity material was the only biological sample available for toxicological analysis. The abdominal cavity material along with suspected powder milk was sent to a forensic toxicology laboratory for analysis.

**Results and Conclusion:** Systematic toxicological analysis was done for poisons like metal phosphide and cyanide. Gas Chromatography/Mass Spectrometry (GC/MS) analysis was performed for drugs and pesticides after extraction with the modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique. Toxicological analysis revealed the presence of lambda-cyhalothrin (insecticide) along with phenytoin (an anti-seizure drug that may be used during treatment) in the abdominal cavity material. Lambda-cyhalothrin was also found in powdered milk, which was ingested by the baby girl. Per research, this is the first time a fatal poisoning of lambda-cyhalothrin in an exhumation sample has been reported.

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### **Lambda-Cyhalothrin, Lactogen, Exhumation**

**K16 The Determination of Commonly Encountered Drugs (Pheniramine, Diphenhydramine, Lidocaine, Norketamine, Ketamine, Tramadol, Chlorpheniramine, Dextromethorphan, Pyrimethamine, and Chloroquine) in Biological Specimens Using a Modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Technique**

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**WITHDRAWN**



## K17 Exo-THC: The Impact of Isobars on Cannabinoid Testing for Seized Drug and Toxicology Cases

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**Learning Overview:** After attending this presentation, attendees will understand the potential impact of delta **9-Tetrahydrocannabinol** (D9-THC) isobars on testing of cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS) for seized drug and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for toxicology cases.

**Impact Statement:** This presentation will impact the forensic science community by informing drug chemists and toxicologists on the potential impact of isobars on cannabinoid identification.

**Introduction:** Delta-8-THC (D8-THC) is a minor constituent in cannabis plants, but the extraction and synthesis of D8-THC is increasing with the availability of Cannabidiol (CBD). D8-THC metabolizes in the body following the same metabolic pathway as D9-THC, resulting in Delta-8-hydroxy-THC (D8-OHTHC) and Delta-8-carboxy-THC (D8-COOHTHC) metabolites. D8-THC can be synthesized from CBD in solvent with the addition of catalysts. The conversion process from CBD is unpredictable and can form byproducts that are not found naturally in cannabis. One such minor byproduct found in cannabis vape oil liquid is delta-9,11-THC (exo-THC).<sup>1,2</sup> Cannabinoid isobars have very similar mass spectra and may be difficult to separate chromatographically.

**Objective:** Evaluate possible interferences caused by exo-THC, D8-THC, Delta-10-THC (D10-THC), and Delta-6a,10a-THC (D-6a,10a-THC) with D9-THC target analytes for seized drug analysis of plant material and extracts by GC/MS and toxicology analysis of blood specimens by LC/MS/MS. The D8-THC metabolites D8-OHTHC and D8-COOHTHC were also included for the LC/MS/MS toxicology method.

**Methods:** Seized drug analysis was conducted on an Agilent® 7890 GC with 5977 MS with a HP-5MS (30m X 0.25mm I.D., 0.25µm film) column with helium at a constant flow of 2mL/min. The temperature program began at 255°C, held for 3.5min, ramped at 30°C/min to 280°C and held for 0.9min for a total run time of 5.23min. Extract analysis included a post run at 325°C of 5min.

Toxicology analysis was conducted on a Shimadzu® Prominence XR LC system with a Sciex® 3200 QTrap tandem MS and a Phenomenex PS C18 2.6µm (50 X 2.1mm) column at 50°C. An eight-minute gradient LC program at 0.6mL/min was utilized with mobile phase A (LC/MS water) and mobile phase B (LC/MS methanol) both with 0.1% formic acid. The gradient program is presented in Table I. The calibration range was from 1 to 100ng/mL for D8-THC, D9-THC, D10-THC, CBD, CBN, and delta-9-hydroxy-THC, and 5 to 500ng/mL for delta-9-carboxy-THC. Stable isotope internal standards were used for all analytes except D10-THC, which used D8-THC-d9.

**Results:** The seized drug GC/MS method was able to chromatographically resolve all cannabinoids listed above. Baseline resolution was not obtained for D6a,10a-THC with D9-THC, which would preclude quantitative or semi-quantitative analysis of D9-THC if D6a,10a-THC was also present. Seven cannabinoid vape oil liquids submitted for analysis containing D8-THC were evaluated for the presence and relative abundance of exo-THC. Exo-THC was a minor component and had less than a 5% relative abundance compared to D8-THC.

For toxicology analysis by LC/MS/MS, D8-COOHTHC was not fully resolved from delta-9-carboxy-THC, which may impact accurate quantitation. Although fragmentation was similar, the ratio of monitored ion pairs (Multiple Reaction Monitoring [MRM] ratio) was different and would not result in misidentification. Hydroxy-delta-8-THC co-eluted with hydroxy-delta-9-THC, but yielded a different MRM ratio. This would preclude the identification of either isobar metabolite when present in a mixture. Exo-THC co-eluted with D9-THC and had an identical MRM ratio, which would lead to misidentification if present alone and falsely elevated concentrations of D9-THC if present in a mixture. Delta-6a, 10a-THC, and D10-THC co-eluted and had the same mass fragmentation. Delta-8-THC, D9-THC, and D10-THC were resolved sufficiently to enable accurate quantitation.

**Conclusion:** Analysis of vape oil liquids by GC/MS is effective for resolution and identification of cannabinoid isobars that may be difficult to differentiate by LC/MS/MS. Although exo-THC could not be differentiated from delta-9-THC by LC/MS/MS due to its extremely low relative amount in vape oils, it is not likely to be present in any detectable amount in biological specimens. An effective partnership between toxicology and seized drug laboratories can aid in determining the risk of cannabinoid isobars being present in biological specimens that may not be differentiated by LC/MS/MS toxicology analysis.

**Table I. LC Gradient Program**

Time (minutes)	%B
0	60
1	70
2.5	70
4	80
5	80
5.01	100
6.5	100
6.51	60
8	Stop

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**Delta-8-THC, Exo-THC, LC/MS/MS**

## K18 An Unusual Case of Accidental Phosphine Inhalation in Pakistan

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**Learning Overview:** The objective of this work is to highlight the increasing trend of phosphine poisoning in Pakistani society.

**Impact Statement:** This presentation will impact forensic toxicologists by presenting a case study involving suspicious deaths of family members due to ingestion of biryani (rice) poisoned with phosphine. Identification of phosphine in antemortem and postmortem biological specimens is of paramount importance in determining the cause of suspicious deaths, especially in Pakistan.

Phosphide tablets are an easily accessible, cheap poison commonly used as an insecticide and wheat pill poison. Phosphides (aluminum phosphide and zinc phosphide) are commonly used as insecticides and rodenticides. Phosphine is increasingly used as a homicidal and suicidal poison in the Pakistani community.

A family consisting of five children and their aunt, a resident of Baluchistan, traveled from Quetta, Pakistan, to Karachi, Pakistan, via train. After their arrival in Karachi, they stayed at a rest house and ate biryani (rice) purchased from a nearby restaurant. After eating biryani, they slept. In the early morning, all children were found unconscious and their aunt had severe vomiting. All were taken to a nearby hospital. All children died within an hour and their aunt also later expired in the hospital. Their antemortem and postmortem samples were taken and submitted to the Punjab Forensic Science Agency (PFSA) for analysis of suspected poisons.

Samples were extracted using a modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction technique and run on an Agilent® Gas Chromatograph coupled with a Mass Spectrometer (GC/MS) for identification of any suspected drug/poison in the samples submitted. To rule out phosphine poisoning, samples were aliquoted into headspace vials and a small amount of zinc dust along with 2mL of 6.0 N sulphuric acid was added. These vials were then crimped and heated in the oven at 70°C for 5 minutes, then 0.5mL of headspace was manually injected into Gas Chromatograph/Nitrogen Phosphorous Detector (GC/NPD) equipment.

GC/MS analysis did not PROVIDE any clue for suspected poisons in any of the submitted samples. However, phosphine analysis using GC/NPD revealed the presence of phosphine in blood samples of two victims, urine samples of four victims, and stomach contents of one victim.

There is a dire need to regulate the sale, availability, and purchase of this potent poison (phosphine). Moreover, detection of phosphine in urine specimens of all four victims revealed that urine is an ideal sample for toxicological screening. In this particular case, initially, it was suspected that all family members died due to eating rice; therefore, the owner of the hotel from where the rice was purchased was arrested. However, when toxicological analysis discovered the presence of phosphine in antemortem and postmortem samples, further investigation was performed. It was found that the rest house in which the family stayed had been fumigated with phosphine a day before their stay.

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### Phosphine, Poisoning, GC/NPD

## K19 An Analysis of Cannabis Exposure and Oxidative Stress Biomarkers in Exhaled Breath Condensate and Oral Fluid

*Meena Swaminathan, MS\*, Florida International University, Miami, FL; Anthony P. De Caprio, Florida International University, Miami, FL; Anamary Tarifa, Florida International University, Miami, FL*

**Learning Overview:** The goal of this work is to present a Liquid Chromatography/Triple Quadrupole/Tandem Mass Spectrometry (LC/QqQ/MS/MS) method for detecting a variety of cannabis exposure and oxidative stress biomarkers in Exhaled Breath Condensate (EBC) and OF (OF) matrices.

**Impact Statement:** This presentation will impact the forensic science community by presenting a reliable analytical method for detection of major and minor cannabinoids and metabolites in the alternative specimen matrices EBC and OF.

Many states in the United States continue to legalize medicinal and recreational cannabis. As a result, there is a growing need in forensic toxicology to investigate cannabis biomarkers that can differentiate between recent/long-term use and licit/illicit consumption, be sampled with relative ease, and, ultimately, correlate with impairment. Furthermore, clinicians are interested in determining whether inhalation of recreational/medicinal cannabis can exacerbate or provide protection from oxidative respiratory stress following exposure.

EBC and OF are alternative, non-invasive matrices that hold promise for identification of cannabis exposure biomarkers. OF is currently being explored as a matrix for cannabis exposure analysis. However, there are currently no reports on the use of EBC for this purpose, although other drugs have been reported to be detected in this matrix. EBC is an aqueous specimen consisting of condensed water vapor, volatiles, and non-volatile components and has several potential advantages for cannabis biomarker detection, including ease of collection and the presence of a wide variety of volatile and non-volatile analytes, metabolites, and markers of respiratory stress.

In this study, selected cannabis and oxostress analytes were spiked into blank EBC and OF and analyzed by LC/QqQ/MS/MS. A total of 20 major and minor cannabinoids, acid precursors, and metabolites were targeted for analysis based on detection reported in human specimens, compounds identified in recreational and/or medicinal cannabis, and availability of standards. Seven internal standards were included for quantitation analysis. Five commonly monitored respiratory oxidative stress biomarkers were also selected for analysis: 8-isoprostane, 8-oxo-7,8-dihydroguanine, 8-oxo-7,8-dihydro-2'-deoxyguanosine, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol, and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol.

An Agilent® 1290 Ultra High-Performance Liquid Chromatography (UHPLC) coupled to a 6470 LC/QqQ/MS/MS with positive and negative mode Electrospray Ionization (ESI) was utilized for this work. Chromatographic separation was achieved using a Zorbax® 120 EC-C18 column (3.0 x 100mm, 1.8µm) and a step gradient elution with an aqueous mobile phase (A) of 5mM ammonium formate with 0.1% formic acid in water and an organic mobile phase (B) of 0.1% formic acid in 75% acetonitrile and 25% methanol. ESI parameters were optimized to the mobile phase composition of choice, and dMRM parameters including MRM transitions, collision energy, and fragmentor voltage were optimized to effectively analyze both the cannabis and oxidative stress biomarkers in a single run time of 26 minutes.

The method was successful in identifying all analytes, including the difficult to separate  $\Delta^8$ -THC,  $\Delta^9$ -THC, cannabidiol, cannabichromene, and cannabicyclol. Limit of Detection (LOD) and Limit Of Quantitation (LOQ) for the target cannabinoids and oxidative stress biomarkers ranged from 1 to 10ng/mL and 7 to 18ng/mL, and from 0.13 to 54ng/mL and 0.38 to 164ng/mL, respectively. Future studies will utilize the method to explore cannabis exposure and oxidative stress biomarkers in a human cohort of cannabis smokers with different exposure characteristics.

### Cannabinoids, Exhaled Breath Condensate, Oxidative Stress

## K20 A Quantitative Analysis of Cannabinoids in Exhaled Breath Aerosols by High-Performance Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry (HPLC/ESI-MS/MS)

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**Learning Overview:** This presentation will describe the process of establishing methods to quantify cannabinoids in exhaled breath aerosols.

**Impact Statement:** This presentation will impact the forensic science community by raising and answering critical questions about how to accurately measure cannabinoids in exhaled breath aerosols. Reliable methods to determine recent use are a critical need for law enforcement.

With the rise in states decriminalizing adult recreational cannabis, there is a need to understand how to measure cannabinoids in biological matrices, and how those concentrations may be related to intoxication. Studies that try to correlate intoxication to cannabinoid concentrations have investigated cannabinoids present in *OF*, sweat, urine, and blood.<sup>1</sup> However, a link to delta9-Tetrahydrocannabinol ( $\Delta^9$ THC) and its metabolites, 11-OH- $\Delta^9$ THC and 11-COOH- $\Delta^9$ THC, has yet to be established. Unlike ethanol, which is abundant in breath, and reliably quantified in a single exhalation, cannabinoids are sparse in breath. However, cannabinoids are lipid-soluble and can be exhaled in aerosols after inhalation of cannabis products. Devices designed to capture breath aerosol particles have been manufactured and breathing maneuvers to increase aerosol production have been investigated.<sup>2,3</sup> Chronic cannabis users can give  $\Delta^9$ THC negative breath samples prior to smoking, and  $\Delta^9$ THC levels increase, then gradually decrease after smoking, where the window of  $\Delta^9$ THC detection can be up to three hours after consumption.<sup>4,5</sup> A recent study tried to correlate  $\Delta^9$ THC concentrations in the breath to blood after smoking, but inter-individual variability needs to be addressed, such as the variability in the amount of particles that one person can produce versus another.<sup>5</sup>

This pilot study investigated the presence of cannabinoids in exhaled breath aerosols collected with an impaction filter device consisting of a wide mouthpiece followed by three parallel collectors. Aerosols were collected prior to and post *ad libitum* consumption of smoked cannabis according to a National Institute of Standards and Technology (NIST) and University of Colorado-approved Institutional Review Board (IRB) protocol. Participants were instructed to follow a low-lung-volume breath hold breathing maneuver for 12 exhalations to increase aerosol production.<sup>3</sup> Breath samples were collected at a baseline session, prior to use, and approximately one hour post use. Blood cannabinoids were monitored to verify compliance with study protocols. One strength of this study was the innovative observational design developed at the University of Colorado, in which experienced cannabis users consume a specific legal market product formulated directly by the dispensary. While initial breath samples were screened for ten cannabinoids and metabolites, this presentation will focus on quantitation of  $\Delta^9$ THC, 11COOH- $\Delta^9$ THC, and cannabinol (CBN, a degradation product of  $\Delta^9$ THC). Method development for the quantification of un-derivatized cannabinoids in breath samples by HPLC/ESI-MS/MS, including analyte extraction, concentration, and the use of process-matched calibrators will be described. Limits of quantification of cannabinoids down to hundreds of pg/mL were achieved with sensitive multi-reaction monitoring and MS/MS, but varied by molecule. Inter-individual variability is also discussed, as well as the presence of 11COOH- $\Delta^9$ THC in some samples, not previously observed in other breath studies.<sup>4,6,7</sup>

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### Cannabis, Breath Aerosols, Mass Spectrometry

## K21 A Sensitive Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method for the Simultaneous Quantification of 32 Drugs in Oral Fluid

*Kayla Marschke, BS, John Jay College of Criminal Justice, New York, NY; Marta Concheiro, PhD, John Jay College of Criminal Justice, New York, NY*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how to extract and quantify 32 drugs in *OF* in driving under the influence cases by LC/MS/MS.

**Impact Statement:** This presentation will impact the forensic science community by providing a new analytical tool for the fast and sensitive detection of 32 drugs in *OF* including cannabis, opioids, stimulants, and benzodiazepines.

Due to the increasing interest by legislature and law enforcement for roadside *OF* testing, the need for toxicology laboratories to develop for methods of confirmation in *OF* has risen. *OF* analysis offers benefits to roadside testing and Driving Under the Influence of Drugs (DUID) cases investigation because of the quick, non-invasive sample collection and the correlation to blood as being a filtrate of it.

A sensitive method was developed for the simultaneous detection of a panel of 32 drugs in *OF* collected by the Quantisal<sup>®</sup> device. The analysis was performed by LC/MS/MS. The target drugs chosen for the method were based on the recommendations by the Toxicology Subcommittee of the National Institute for Standards and Technology's (NIST's) Organization of Scientific Area Committees (OSAC) for DUID cases. Additional substances were included based on the drug prevalence in the New York City region. The method tested for the following drugs: delta-9-Tetrahydrocannabinol (THC), amphetamine, methamphetamine, 3,4-Methylenedioxymethamphetamine (MDMA), 3,4-Methylenedioxyamphetamine (MDA), cocaine, benzoylecgonine, cocaethylene, carisoprodol, meprobamate, zolpidem, alprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, codeine, 6-monoacetylmorphine (6-AM), buprenorphine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, oxymorphone, tramadol, ketamine, and Phencyclidine (PCP).

Quantisal<sup>®</sup> collection devices collect 1mL of sample *OF*, for a 1:4 dilution in buffer. In the analysis, this study employed 1mL of the *OF*-buffer mixture, which contains 0.25mL of neat *OF*. The *OF*-buffer samples were prepared by Solid Phase Extraction (SPE) using Strata<sup>™</sup> X 33um polymeric reversed phase SPE cartridges. Samples were eluted using dichloromethane: 2-propanol (75:25, v/v) and treated with 1% hydrochloric acid in methanol before evaporation to avoid amphetamines loss. The samples were reconstituted using 0.1% formic acid in water: acetonitrile (95:5, v/v) and injected into the LC/MS/MS.

LC/MS/MS in positive electrospray mode was used for the detection of drugs in *OF*. Two Multiple Reaction Monitoring (MRM) transitions were acquired for each analyte. The mobile phases employed were 0.1% formic acid in water (A) and acetonitrile (B). A reversed phase chromatographic column, Kinetex<sup>®</sup> C18 100 x 2.1 mm with particle size 1.7um, was used for separation, and the total run time was 15min. The Limits Of Quantification (LOQ) ranged from 0.5 to 5ng/mL, depending on the compound. LOQ was 0.5ng/mL for fentanyl, amphetamine, methamphetamine, MDMA, cocaine, cocaethylene, carisoprodol, meprobamate, zolpidem, tramadol, ketamine, and PCP; LOQ was 1ng/mL for THC, alprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, and methadone, 6-acetylmorphine; 2ng/mL for buprenorphine; 5ng/mL for codeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone, benzoylecgonine, and MDA. The linearity range was from the LOQ to 500ng/mL. The method is currently being fully validated following the recommendations of Academy Standards Board. This method can pose useful for toxicology labs planning to add *OF* screening and confirmation as states expand roadside *OF* testing.

### Oral Fluid, LC/MS/MS, DUID

## K22 The Detection of Gabapentin in the Human Umbilical Cord

*Amy Racines, MS, USDTL, Des Plaines, IL; Andre Sukta, MSFS\*, USDTL, Des Plaines, IL; Joseph Jones, PhD, USDTL, Des Plaines, IL*

**Learning Overview:** After attending this presentation, attendees will have learned about the prevalence of gabapentin abuse within the neonatal population.

**Impact Statement:** This presentation will help the community appreciate the prevalence of gabapentin abuse in the neonatal population.

**Background/Introduction:** Gabapentin is an anti-convulsant and is used to treat neuropathic pain. It is a less-addictive alternative to opioids; however, abuse and addiction still occur and have been increasing since the early 2000s. Gabapentin has been shown to cross the placenta and accumulate in the fetal compartment, resulting in a higher rate of pre-term births, lower birth weights, and neonatal abstinence syndrome. Early identification of newborns exposed to gabapentin *in utero* is vital for proper treatment and better neonatal outcomes.

**Objective:** The objective of this study was to develop and validate a method for the detection of gabapentin in umbilical cord tissue using a Laser Diode Thermal Desorption/Tandem Mass Spectrometry (LDTD/MS/MS) screening analysis and a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) confirmation analysis.

**Methods:** Umbilical cord (0.5grams) in acetonitrile was homogenized in a NextAdvance Bullet Blender™ Homogenizer. The specimens were centrifuged, and the supernatants were subjected to a solid phase extraction procedure. The dried extracts were reconstituted with a methanol:deionized water solution containing 100µg/mL of EDTA. Extracts were then spotted into a LazWell™ 96 Plate and dried prior to analysis on the LDTD/MS/MS. Presumptive positive specimens were confirmed through a second aliquot (0.5g) homogenized in acetonitrile. The supernatant was filtered prior to analysis by LC/MS/MS.

**Results:** All validation criteria followed the American National Standards Institute/ Academy Standards Board (ANSI/ASB) Standard 036, First Edition, Standard Practices for Method Validation, published in 2019. The LDTD/MS/MS analysis had a cutoff of 10ng/g, with a limit of detection of 3ng/g. Both the extracts and dried LazWell™ plates were stable for 48 hours, and no carryover or obvious interferences were observed during the validation. Significant ion suppression, however, was observed. Authentic specimens ( $n=70$ ) were analyzed, and presumptive positive specimens were forwarded to LS/MS/MS for confirmation.

The confirmation method was validated with a cutoff of 10ng/g and a limit of detection of 0.2ng/g. Precision and accuracy was challenged at 4ng/g, 12.5ng/g, and 100ng/g, and results were acceptable. Extracts were stable for up to seven days, and no obvious interferences, significant matrix effect, or carryover were observed. Linear range was determined to be between 4ng/g and 50ng/g. All presumptive positive authentic specimen analyzed ( $n=3$ ) showed 100% correlation with the screening analysis.

**Discussion/Conclusion:** An increasing number of newborns have been exposed to gabapentin *in utero*, and a crucial aspect of helping these babies recover is early identification. The validated method described here allows early and accurate identification of prenatal exposure to gabapentin, allowing for early intervention and more successful outcomes.

### Gabapentin, Umbilical Cord, LDTD

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## K23 Comparative Biotransformation of New Psychoactive Substances by Human Liver Microsomes, Electrochemical Oxidation, and *In Silico* Analysis

Leonardo Maya, BS\*, Florida International University, Miami, FL; Anthony DeCaprio, PhD, Florida International University, Miami, FL

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**Learning Overview:** Attendees will obtain a better understanding of the biotransformation of selected New Psychoactive Substances (NPS) via different *in vitro* methods.

**Impact Statement:** This research explores alternative *in vitro* metabolomic approaches to assist in the identification of appropriate metabolites for NPS monitoring in forensic toxicological screening and confirmation.

NPS refers to a category of newly modified or synthesized drugs designed to mimic Central Nervous System (CNS) pharmacological effects associated with more classical illicit substances. Using simple or complex changes to core structures, synthesis of NPS can result in a variety of physiological effects as well as yielding a wide array of metabolic products. Human Liver Microsome (HLM) studies are one of the most common *in vitro* assays performed to predict phase I metabolism of drugs. However, these may not encompass the full panel of metabolites that may be generated in human subjects. To better understand and develop metabolomic data for NPS, other methods must be explored.

This study takes a multidimensional approach on phase I metabolism of two synthetic cannabinoids, JWH-018 and 5F-APINAC. Utilizing traditional HLM, Electrochemical Oxidation (ECO), and *in silico* tool (MetaSite), phase I metabolism was assessed. Previous published data has shown metabolomic spectra of the older generation NPS JWH-018 via HLM. However, there is little information regarding the *in vitro* metabolism profiles of 5F-APINAC via HLM, and essentially none for ECO and *in silico* methods. Also, the efficacy of the various *in vitro* methods featured in this study have yet to be comparatively assessed.

The metabolic assays conducted in this study allowed for the screening and confirmation of parent JWH-018 and 5F-APINAC as well as their metabolites. A total of ten metabolites were produced for JWH-018. The *in vitro* assays performed on 5F-APINAC yielded a total of 15 metabolites. Metabolites were categorized based on mono-, di-, trihydroxylation, and dealkylation, among other phase I biotransformations commonly seen in *in vivo* studies. Results initially produced via HLM were compared with the ECO methods and MetaSite data. Through this comparative assessment, metabolites similar to those previously found in literature were matched, along with the discovery of several metabolites not previously reported. These results suggest that ECO and *in silico* analysis of oxidative metabolites may be useful complements or alternatives to HLM for comprehensive profiling of NPS metabolites in forensic toxicology.

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### Forensic Toxicology, New Psychoactive Substances (NPS), *In Vitro* Biotransformation



## K24 Method Development for Drug Court Samples

Kourtney Steen, MSFS\*, University of Alabama at Birmingham, Birmingham, AL; Elizabeth Gardner, PhD, University of Alabama at Birmingham, Birmingham, AL

**Learning Overview:** After attending this presentation, attendees will better understand the Jefferson County Drug Court program, the extraction method utilizing DPX® tips, the data related to emerging drugs, and identifying certain drugs through two derivatizing agents.

**Impact Statement:** This presentation will impact the forensic science community by providing mass spectral data, discussing the importance of derivatization to separate co-eluting compounds, and an extraction method that uses DPX® tips to conduct Solid-Phase Extraction (SPE) manually.

This project is in collaboration with the Jefferson County Drug Court system. The Drug Court program is available in Jefferson County, AL, for youths and adults to help offenders with felony drug offenses. Individuals can have their cases dismissed once they complete the rehabilitation program. There are three phases to the program in which random drug tests are conducted through a color code system where they are being screened weekly, bi-weekly, and monthly.<sup>1</sup> The program is completed once the individual has received a clean drug test for 90 days. The objectives of this project were: (1) ongoing random confirmation of drug court urine results, and (2) identifying new drugs as they enter the community.

The method used to identify drugs in the urine samples provided was through SPE, using commercially available pipette tips loosely packed with solid-phase sorbent contained by two solvent permeable frits (DPX® tips). DPX® tips are generally used for high-volume robotic systems.<sup>2</sup> In this case, they are used manually, as one extraction method that can be used for acidic, basic, and neutral drugs.

Urine samples were loaded onto the sorbent and rinsed with distilled water (d-H<sub>2</sub>O). Neutral and acidic drugs were extracted with acetonitrile. Basic drugs were extracted with dichloromethane:isopropyl alcohol: NH<sub>4</sub>OH (78:20:2). The extracts were dried under Nitrogen (N<sub>2</sub>), dissolved in ethyl acetate:methanol (80:20), and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Derivatization was necessary for certain drugs. Derivatization is used to separate co-eluting compounds. It helps to create a better chromatography with sharper peaks, larger masses, and often longer retention times. Opioids, benzoylecgonine, and THC-COOH were derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). Amphetamines were derivatized with Heptafluorobutyric Acid (HFBA)<sup>3</sup>.

The Jefferson County Drug Court screens the urine samples for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, fentanyl, methadone, heroin, buprenorphine, and creatinine. As part of the method development, 22 drug court samples were analyzed. Of the samples, 11 were negative for drugs of abuse. Of the remaining half, presumptive testing by the Drug Court found most of the samples were positive for opiates, benzodiazepines, amphetamines, cannabinoids, cocaine, and fentanyl. With the analysis of the mass spectra, this study confirmed the positive cases. This study was also able to find drugs not identified by the current Drug Court panel, including xylazine and escitalopram. Xylazine is a veterinary anesthesia. Escitalopram is a Selective Serotonin Reuptake Inhibitor (SSRI).

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2. Dayanne Cristiane Mozaner et al. Disposable pipette tips extraction: Fundamentals, applications and state of the art. *Journal of Separation Science* 39.6 (2016): 1168-1172.
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### Solid-Phase Extraction, GC/MS, DPX Tips

## K25 The Determination of 67 Analytes in Oral Fluid by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** This presentation will discuss the validation, benefits, and limitations of a precise and accurate method for the quantitative determination of 67 analytes in *OF* using LC/MS/MS. These analytes include benzodiazepines, amphetamines, opiates, opioids, stimulants, hallucinogens, THC, cotinine, antidepressants, opiate agonists, muscle relaxants, anticonvulsants, antipsychotics, and antitussives.

**Impact Statement:** This presentation will impact the forensic science community by displaying a validated method and linear range for drug testing various clinical and forensic analytes in *OF*. There is a wide array of analytes tested in this method over various drug classes.

**Hypothesis/Proposition/Objective:** This method was developed and validated to expand the laboratory's *OF* testing menu.

**Methods:** A 96-well solid phase extraction procedure was used to extract 300 $\mu$ L of *OF* samples consisting of 1:3 neat *OF*: Quantisal™ buffer. Samples were spiked with a deuterated internal standard mix prior to extraction. After wash steps and drying the columns, the analytes were eluted with elution solvent into a 96-well collection plate. After evaporating the eluent to dryness, samples were reconstituted in an aqueous solution of 3% methanol in water. The samples were injected into an LC/MS/MS to separate the analytes based upon their solubility in the stationary and mobile phases. The column effluent was directed into heated electrospray ionization source, which ionizes the analytes as they elute. During the method development stage, all analytes were infused using the molecular weight of each compound in positive ionization mode as the precursor ion. These pre-selected precursor ions were selected in the first quadrupole and fragmented in the second quadrupole (collision cell) by an inert gas collision. The fragment ions were selected in the third quadrupole. The combination of retention time, precursor/product masses, and fragment pattern allowed for the qualitative identification of the analytes. The area of each non-deuterated analyte to deuterated analog ratio permits for the quantification of each analyte. The ratios are compared to a six-point calibration curve that brackets each analyte cutoff and demonstrates the linear range of the assay.

**Summary of Results:** This method was validated for quantitative results of all 67 analytes that includes four analytical measurement ranges varying from 0.5–25ng/mL, 1–50ng/mL, 2.5–125ng/mL, and 12.5–625ng/mL. The calibration curves had coefficient of determination values greater than 0.99 to determine the linear range. The accuracy showed the results of each calibrator were within the 80–120% of the target concentration after 20 injections per calibrator. The precision study has five concentrations that were tested at 75% of the cutoff, 125% of the cutoff, 2 times the cutoff, 40% of the Upper Limit Of Linearity (ULOL), and 80% of the ULOL.<sup>1</sup> No analytes had a percent accuracy deviating outside 80–120% after 40 injections of each concentration were examined. Carryover of the method was determined for each analyte by 20 injections of the highest concentration calibrator followed by a blank injection and was less than 2%. During the analyte interference study, the percent recovery was accepted if it was within 90–110% of the target. This study showed that pregabalin has major ion suppression when pseudoephedrine is present. Normalized matrix factor equation was used to determine that no biological matrices affected this method.

### Reference(s):

<sup>1</sup> ANSI/ASB Standard 036, First Edition Standard Practices for Method Validation in Forensic Toxicology. (2019). *AAFS Standard Boards*.

### Toxicology, LC/MS/MS, Oral Fluid

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## K26 When Being Positive Is Enough—Unlocking Child Abuse in a Non-Disclosing Child Via Toxicological Hair Testing: A Case Study

*Jessica Volz, DNP\*, Adventist HealthCare Shady Grove Medical Center, Rockville, MD*

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**Learning Overview:** After attending this presentation, attendees will understand how toxicology testing of hair and collaboration among disciplines in cases of non-verbal/non-disclosing children suspected of being abused or neglected can provide critical information for safety planning and legal proceedings.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how toxicology testing of hair can be utilized to support child safety through the elimination of plausible and innocent explanations, even when expert opinions differ.

This case study is of a non-verbal/non-disclosing child suspected of being sexually abused by a secondary relative and whose hair toxicology testing revealed ingestion of cocaine. This testing was utilized as a litigation tool to eliminate argument leading to child protection intervention that would have not been otherwise possible.

Toxicological testing of hair samples is not often used in the acute hospital setting. The limitations of this testing are generally cited as the primary reason for this. However, there is some evidence to support consideration of this testing after the age of 34 months.<sup>1</sup> Though toxicological testing of hair may have significant limitations, this case study supports the argument that there is a time and place for it when investigating cases of potential abuse or neglect in non-verbal or non-disclosing children.

In this case, a non-disclosing preschool-aged child with an unremarkable physical examination presented in the hospital setting for a child sexual abuse examination. Despite the lack of findings in the physical examination, the clinician sent blood, urine, and hair for toxicology testing. Blood and urine testing returned with no substances detected. After a negative hair wash, testing of the hair shaft revealed the child's hair was positive for cocaine and benzoylecgonine.

Consultation between two toxicologists and the hospital clinician concluded that there were differing opinions on the number of exposures and the amount of cocaine ingested to produce this result. There was consensus that it could not be determined if the ingestion was intentional. However, the presence of cocaine and the metabolite benzoylecgonine indicated that the child had ingested cocaine and was at minimum supportive of neglect. Superficially, this opinion might be dismissed as useless due to its ambiguity. However, despite this challenge, the science supported the opinion that cocaine and benzoylecgonine were present. For this reason alone, it was cited by the primary litigator as the linchpin that resulted in a settlement that was in the best interest of the safety of the child.

### Reference(s):

- <sup>1</sup> Alvarez, J.C.; Lasne, L.; Etting, I.; et al. Hair analysis does not allow to discriminate between acute and chronic administrations of a drug in young children. *Int J Legal Med* 132, 165–172 (2018). <https://doi.org/10.1007/s00414-017-1720-5>.

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### Toxicology Testing, Child Abuse, Cocaine

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## K27 Electrochemical and Density Functional Theory (DFT) Studies for Fentanyl and Fentanyl Analogs

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**Learning Overview:** This presentation will describe the development and application of electrochemical approaches for the presumptive determination of fentanyl and fentanyl analogs, as well as the computational analysis with DFT. Information provided will include the optimized detection methods, the comparison between experimental and calculated spectra, and the data analysis for multiple analytes.

**Impact Statement:** This presentation will impact the forensic community by demonstrating the electrochemical approaches in the presumptive screening of fentanyl analogs and opioids. The new methods can rapidly distinguish opioids and fentanyl analogs.

The abuse of opioids has been a critical issue to the public health for years. With the appearance of new fentanyl analogs, the overdose rates of opioids continue to bloom. Current screening methods, such as immunoassay, have difficulty detecting the full range of opioid analogs due to a wide variety of structural variations. This study has been working on alternative screening methods. Electrochemical approaches, including square wave voltammetry and cyclic voltammetry, quickly distinguish the group of fentanyl analogs from other opioids with lower cost and easier operations. Both screening approaches are convenient for point-of-care analysis and laboratory tests.

The electrochemical approaches utilize the Screen-Printed Electrodes (SPE) and Phosphate Buffered Saline (PBS) buffer. Solid drugs were dissolved in the buffer solutions, then dropped on electrodes and analyzed via a CHI instrument with an energy range of 0.5–1.8eV. The **Square Wave Voltammetry (SWV)** spectra identify fentanyl analogs from mixtures with the optimized condition for individual drugs, such as fentanyl, cocaine, heroin, opioids, amphetamines, and adulterants. Electrochemical approaches are less sensitive than instrumental methods, however, its operation and preparations are easier for untrained staff. To understand the cross-reaction of fentanyl analogs and impurities as well as the metabolites, DFT calculations displayed the energy changes and pathways from parent drugs to metabolites.

The electrochemical method permits a rapid, easily operated presumptive test for opioids with different benefits. When the method is coupled with other screening methods, such as surface-enhanced Raman spectroscopy as was done in the laboratory, the method can be orthogonal to mass spectrometry and sufficiently sensitive to detect compounds at toxicologically relevant levels. As a result, it should be particularly useful for the screening of opioids and other novel psychoactive substances.

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### Fentanyl Analogs, Surface-Enhanced Raman Spectroscopy, Square Wave Screening Test

## K28 The Stability of Ethanol in Oral Fluids Samples Using the Quantisal® Collection Device

*Mairin Higgins, BS\*, Arcadia University, Glenside, PA; Kelvin Barnes, BS, Immunalysis, Ponomo, CA; Heather Harris, MFS, JD, Arcadia University, Glenside, PA; Karen Scott, PhD, Arcadia University, Glenside, PA*

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the stability of ethanol in *OF* samples, collected using the Quantisal® collection device, in different temperature environments after several months of storage.

**Impact Statement:** This presentation will impact the forensic science community by determining the most optimal temperature and storage environment for the stability of ethanol in *OF* samples.

Ethanol is the most widely used drug in most societies. Driving under the influence of alcohol is a problem internationally that can be deterred by random roadside testing. Ethanol, a Central Nervous System (CNS) depressant, negatively impacts an individual's ability to drive by causing poor reaction times, causing lapses in judgement and impairing motor coordination. Blood has been the preferred matrix for drug and alcohol testing, but collection is an invasive process and results in delays in obtaining samples. *OF* is an alternative testing matrix with a rapid turnover and a non-invasive collection technique. Concentrations of ethanol in *OF* are comparable to ethanol concentrations in blood once equilibrium has been reached.

The Quantisal® collection device is used for collecting *OF* samples to be used for *OF* drug testing. This device utilizes a collection pad to be placed under the tongue to collect *OF*. The collection devices can be used anywhere to collect approximately 1mL ( $\pm 10\%$ ) of *OF* for screening, confirmation, and repeat testing. The collection devices utilize approximately 3mL of buffer to stabilize drugs that may be within the *OF* collected.

This research aims to determine the impact of temperature environments, storage environments, and authenticity of samples on stability of ethanol through monitoring the concentration of ethanol over a six-month time span. Temperature environments include a freeze/thaw cycle, a freezer, refrigerator, room temperature, and oven set to 37°C. Storage environments include samples retained within the Quantisal® device as well as samples transferred to Eppendorf® tubes and glass tubes with stoppers. Both spiked and authentic samples were used to study stability.

Ethanol-positive samples, both spiked and authentic, were analyzed in triplicate using Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS) in parallel. ELISA offers high-sensitivity, rapid analysis of samples. HS/GC/MS instrumentation was used for confirmation of ethanol concentrations in spiked and authentic samples. Temperatures in each environment were monitored using a temperature logger. The oven temperature was held at 37°C, the room temperature was an average of 22°C, refrigerator temperature was found to be an average of 2°C, and the freezer environment was -12°C on average.

Samples were tested at set time increments over a six-month span:  $t_0$ ,  $t_{\text{day } 1}$ ,  $t_{\text{day } 2}$ ,  $t_{\text{day } 3}$ ,  $t_{\text{day } 7}$ ,  $t_{\text{day } 14}$ ,  $t_{\text{day } 30}$ , and every 30 days for the remaining time frame. Storage temperature of the samples impacted the stability of ethanol in samples. Stability of samples remained fairly constant over the first four testing times, regardless of temperature environment. Parafilm was effective at sealing Eppendorf® tubes in an elevated temperature environment to keep ethanol concentrations stable. Samples stored in glass tubes with rubber stoppers remained stable for a longer period than those in plastic Eppendorf® tubes. Samples stored in cooler environments were more stable than those at room temperature and in the oven.

### Stability, Oral Fluid, Ethanol

## K29 The Development of a Method for the Extraction and Analysis of Fentanyl and Alprazolam From Dermestid Beetles, Larvae, Casings, and Frass

Stephanie R. VanLysebettens, BA\*, Arcadia University, Glenside, PA; Stephanie Rainer, MSFS, Rutgers University, New Brunswick, NJ; Lorna Nisbet, PhD, Anglia Ruskin University, Cambridge, Cambs, United Kingdom; Karen S. Scott, PhD, Arcadia University, Glenside, PA

**Learning Overview:** Those who attend this presentation will be able to utilize the proposed extraction method followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis to determine the presence of fentanyl and alprazolam in beetles, casings, larvae, and frass.

**Impact Statement:** This presentation will impact the forensic science community by utilizing Dermestid beetles to determine the presence of drugs in decedents who may be void of other tissues for analysis.

Insects play a crucial role in the determination of postmortem intervals of decaying bodies. Extensive studies have been conducted on *Diptera: Calliphoridae*, but little on *Coleoptera: Dermestidae*. Studies on Diptera larva have shown that consumption of a decedent with drugs in their system transfers that drug into the insect.<sup>1-6</sup> The ability to use Dermestid beetles comparably to blow flies would allow evidence to be obtained from a carcass much later in the cycle of decay as beetles do not arrive until approximately nine days after death.

Drugs focused on in this study included fentanyl and alprazolam. These drugs were chosen because of the increasing opioid epidemic in the United States and around the world. Alprazolam is a benzodiazepine that is often used in conjunction with opioids to heighten effects. As benzodiazepines are Central Nervous System (CNS) depressants and fentanyl is an opioid analgesic, the combination can cause respiratory depression and an increased death rate.

Prior to the study a main colony was developed starting with 500 larvae containing an unknown combination of *D. ater*, *D. lardarius*, and *D. maculatus*. From this colony, three replicates were pulled, each containing four separate breeding colonies. Drugs were introduced to the beetles through a food matrix consisting of an 18:1 ratio fish meal and brewer's yeast supplemented with 10% casein powder. Breeding colonies were fed a negative control or food matrix spiked with one of the two drugs of interest. Homogenization using a Biotage® OMNI Bead Ruptor 24 and wash procedures of the beetles, casings, larvae, and frass were performed prior to extraction using Solid-Phase Extraction (SPE). All samples were injected into a SCIEX™ API 3200 LC/MS/MS with Shimadzu® HPLC system. An Agilent® InfinityLab Poroshell 120 EC-C18 column was used (3.0 x 100mm, 2.7-Micron, 1,000bar with Column ID). Product ions for alprazolam were monitored at 281.3 and 205.2 m/z and for fentanyl at 188.1 and 105.0 m/z. Deuterated internal standards for both drugs were also used.

Both drugs were successfully detected from the various matrices collected and in some cases, a dose-dependent response was observed. Dermestid beetles and their associated waste products are useful toxicological tools when conventional samples are no longer available due to adverse conditions at the scene of a drug-related death.

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### Dermestidae, Fentanyl, Alprazolam

## K30 Validation of a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method for 23 Fentanyl Analogs in Oral Fluids

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**Learning Overview:** The goal of this presentation is to demonstrate an LC/MS/MS method development and validation study for analyzing whole blood and OF for 23 fentanyl analogs. Detection limits between the two matrices will be compared.

**Impact Statement:** This presentation will impact the forensic science community by showing the value of OF as a non-invasive toxicologically important matrix when other matrices are not available or when non-invasive sample collection is preferred.

Currently, synthetic opioids, including fentanyl analogs, are the leading cause of overdose deaths in the United States.<sup>1</sup> New fentanyl analogs are being made regularly and are used to avoid classification as illegal, policy restrictions on manufacturing and/or detection in standard drug tests.<sup>2</sup> Fentanyl analogs are drugs designed to mimic the pharmacological effects of fentanyl. Fentanyl is a highly potent pain reliever that produces effects such as euphoria, drowsiness, sedation, pain relief, etc.<sup>3</sup>

Due to the small detection window in OF, blood or urine samples are often utilized in forensic laboratories, but the benefit of testing OF is that it requires non-invasive sample collection and is not easily contaminated.<sup>4</sup> In this study, a rapid LC/MS/MS method was developed and validated for analyzing OF for 23 fentanyl analogs. Samples were prepared by phospholipid depletion and protein precipitation using 200µL of either whole blood or OF and 1% acetic acid in acetonitrile. Separations were completed using an Agilent® ZORBAX® Eclipse XDB-C18 (2.1x100mm) with a Vanquish™ Ultra High-Pressure Liquid Chromatography (UHPLC) System. Gradient elution was performed with 0.1% formic acid in water and acetonitrile. Identification and quantitation were conducted with a TSQ Endura™ Triple Quadrupole-Mass Spectrometer (QqQ-MS) operating in selective reaction monitoring mode. This LC/MS/MS technique combines low complexity sample preparation with the selectivity and sensitivity of LC/MS/MS.

This method was validated in whole blood and OF. Validation studies consisted of precision, accuracy, ion suppression/enhancement, analyte recovery, analyte stability, dilution integrity, matrix interferences, and cross-panel interferences. Limit Of Quantitation (LOQ) are typically 0.05ng/mL with an analytical measurement range reaching 20ng/mL. Validation results show that analytes are detected in OF at significantly lower LOQs than can be achieved in whole blood.

This method allows for the detection of fentanyl analogs in OF. This can help to determine overdose cases and can be implemented in routine drug testing or Driving Under the Influence (DUI) cases. With the constant rapid increase of overdose cases involving fentanyl analogs, it is important to have a quick and efficient way of detecting opioids in various matrices.

### Reference(s):

1. *Understanding the Epidemic* (2021). Centers for Disease Control and Prevention. <https://www.cdc.gov/opioids/basics/epidemic.html>.
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### LC/MS/MS, Fentanyl Analogs, Oral Fluid

## **K31 An Investigation of Structural Features on the Binding of Synthetic Cannabinoids to the CB1 Receptor: A Computational Study of the JWH Compounds**

*Krishna Chaturvedi, PhD, The University of Mississippi, University, MS; Caroline Spencer, PhD, STI Electronics Inc., Madison, AL; Pankaj Pandey, PhD, The University of Mississippi, University, MS; Robert Doerksen, PhD, The University of Mississippi, University, MS; Murrell Godfrey, PhD\*, The University of Mississippi, University, MS*

**Learning Overview:** After attending this presentation, attendees will understand the interactions between JWH synthetic cannabinoids and the active-state CB1 receptor.

**Impact Statement:** This presentation will provide a better understanding of key features of JWH compounds with specific CB1 receptor residues through molecular docking and all-atom molecular dynamics simulation. The knowledge of these critical interactions and structural features of JWH compounds will help forensic chemists to design and predict novel structures of JWH synthetic cannabinoids.

To date, there have been two cannabinoid receptors identified, CB1 and CB2. The cannabinoid receptor 1 is the most abundant G protein-coupled receptor present in the Central Nervous System (CNS) and is also found in the immune system and peripheral organs.<sup>1</sup>  **$\Delta^9$ -Tetrahydrocannabinol** ( $\Delta^9$ -THC) and synthetic cannabinoids interact with CB1 receptors and cause “psychoactive effects,” functioning as CB1 receptor agonists. JWH compounds and other synthetic cannabinoids are seen under the brand names of “Spice,” “K2,” “herbal incense,” “Cloud 9,” and many more. They have become an important area of research in the forensic science community due to their drug abuse potential and their unpredictable toxicity.<sup>2</sup>

In the present study, an active-state CB1 X-ray crystal structure was used to design novel JWH-virtual compounds by understanding the ligand-receptor interactions between the CB1 receptor and the JWH synthetic cannabinoid family using docking, binding free-energy calculation, and all-atom Molecular Dynamics Simulations (MDs).

The binding free-energy data revealed that the carbonyl group between the naphthalene and the indole and the length of the N-linked alkyl chain were two important structural characteristics that influence the predicted CB1 activity. Increasing the length of the alkyl chain on the naphthalene of JWH-XXX compounds led to better predicted binding affinity with the CB1 receptor. The MD (100ns) analysis results showed that virtually designed new JWH compounds and selected JWH compounds fit well into the active site of the CB1 receptor and formed stable and strong hydrophobic interactions with the key residues: Phe170, Phe174, Phe177, Phe189, Leu193, Phe200, Phe268, and Trp279. Identifying the key interactions between the synthetic cannabinoids and the CB1 receptor is a step toward a better understanding of the effects of these drugs, including toxicity and potential for abuse and the design of new analogs.

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

1. Shim, J.Y.; Bertalovitz, A.C.; Kendall, D.A. Identification of Essential Cannabinoid-Binding Domains Structural Insights Into Early Dynamic Events In Receptor Activation. *J. Biol. Chem.* 2011, 286, 33422-33435.
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### **Synthetic Cannabinoid, CB1 Receptor, Molecular Modeling**



## K32 A Comparison of Meconium, Synthetic Meconium, and Urine by High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) in the Simultaneous Detection of Illicit Drugs and Metabolites

Shannon Palladino, MPS\*, Pennsylvania State University, University Park, PA; William Campbell, PhD, Pennsylvania State University, University Park, PA

**Learning Overview:** Attendees can expect to learn about the value of using meconium in the determination of maternal drug use and/or exposure, extraction procedures used for various biological matrices and the LC/MS/MS method development involving a broad panel of different drugs of abuse. Overall, attendees should gain an understanding of the influence of different matrices (urine, meconium, and synthetic meconium) on LC/MS/MS quantitative analysis of a drug panel of interest to forensics.

**Impact Statement:** This presentation would be novel in its comparison of urine, meconium, and synthetic meconium from a toxicological perspective supported by validated LC/MS/MS methods. Such an evaluation would confirm or deny the viability of synthetic meconium as an alternative to natural meconium, which could potentially reshape how research is conducted on meconium in the future.

The toxicological determination of drugs, environmental toxins, or other agents that can be passed from mother to child *in utero* is of great relevance in clinical and forensic settings, with drugs of abuse being of particular interest for forensic implications. In order to properly treat a neonate possibly suffering from the effects of fetal drug exposure and to propose the correct legal course of action, the substance or substances in question must be determined and quantified. Matrices like blood and urine are commonly used in toxicological practices and studies, but when determining *in utero* drug exposure, meconium has emerged as an ideal biological matrix. Not only does meconium allow for non-invasive collection, but from an analytical perspective, it has a long window for drug accumulation, allowing for detection with higher sensitivity and specificity than most other matrices.<sup>1-3</sup>

However, for research purposes, meconium can be a problematic material to work with due to difficulties in obtaining samples and due to privacy and confidentiality issues. That is why this project will focus on the comparison of meconium to synthetic meconium. Synthetic alternatives to biological matrices pose great opportunities in performing clinical and toxicological assessments, and there has been little research done on its potential as a valid substitute for real meconium.<sup>4</sup>

An LC/MS/MS method has been developed to separate and quantitate a panel of 41 drugs of abuse, including stimulants, opiates, benzodiazepines, barbiturates, cannabinoids, and their metabolites. Because there is scarce information about a direct comparison between meconium analysis and urinalysis—a standard practice in clinical testing—this method will also be applied to urine alongside meconium, synthetic meconium, and water. This research aims, through method validation and statistical analysis, to reach a preliminary conclusion about the comparability of synthetic meconium to natural meconium for use in LC/MS/MS method development and research in comparison to urine as a reference matrix.

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2. Moore, C.; Negrusz, A.; Lewis, D. Determination of Drugs of Abuse in Meconium. *J. chromatogr.* 1998, 713 (1), 137–146.
3. Ostrea, E.M.; Bielawski, D.; Villanueva-Uy, E.; Ager, J. Meconium Analysis—A Promising Tool to Detect Fetal Exposure to Environmental Toxins [https://cfpub.epa.gov/ncer\\_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/2239](https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/2239) (accessed Dec 17, 2020).
4. Haglock-Adler, C.J.; Hurley, A.; Strathmann, F.G. Use of Synthetic Urine as a Matrix Substitute for Standard and Quality Control Materials in the Clinical Assessment of Iodine by Inductively Coupled Plasma Mass Spectrometry. *Clin. Biochem.* 2014, 47 (15), 80–82.

### Meconium, Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), Drugs of Abuse

## K33 Standards Development Activities in Forensic Toxicology

Marc LeBeau, PhD\*, Federal Bureau of Investigation Laboratory, Quantico, VA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of forensic toxicology.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to forensic toxicology. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews published standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in forensic toxicology will be presented. These include:

Recent standards that have been added to the OSAC Registry (ANSI/ASB 017: *Standard Practices for Measurement Traceability in Forensic Toxicology*; ANSI/ASB 036: *Standard Practices for Method Validation in Forensic Toxicology*; ANSI/ASB 053: *Standard for Report Content in Forensic Toxicology*; and ANSI/ASB 037: *Guidelines for Opinions and Testimony in Forensic Toxicology*)

OSAC Proposed Standards:

Published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process (ANSI/ASB 054 *Standard for Quality Control Programs in Forensic Toxicology Laboratories*; ANSI/ASB 119 *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations*; ANSI/ASB 120 *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations*; ANSI/ASB 121 *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigations*)

Documents currently in development by the ASB (ANSI/ASB 055: *Standard for Breath Alcohol Measuring Instrument Calibration*; ANSI/ASB 098: *Standard for Mass Spectral Data Acceptance in Forensic Toxicology*; ANSI/ASB 113: *Standard for Identification Criteria in Forensic Toxicology*; ANSI/ASB 153: *Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories*; ANSI/ASB 156: *Guidelines for Specimen Collection and Preservation in Forensic Toxicology*; ANSI/ASB 152: *Standard for the Minimum Content Requirements of Forensic Toxicology Procedures*)

Documents currently being drafted at the OSAC (*Standard Practices for Evaluating Measurement Uncertainty of Quantitative Measurements in Forensic Toxicology*; *Guidelines for Performing Alcohol Calculations in Forensic Toxicology*; *Standard for Breath Alcohol Instrument Specifications*; *Standard for Education and Training of Forensic Toxicology Personnel*; *Quality Management Systems in Forensic Toxicology Laboratories*; *Standard Method for Blood Ethanol Identifications and Quantitations*; *Standard Practices for Continued Education in Forensic Toxicology Laboratories*; *Standard for the Accreditation of Forensic Toxicology Laboratories*)

Priorities for new documents or work products and other highlights:

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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### Forensic Toxicology, Standards, ASB

## K34 The Rise of the “Cannabisomers”

*Tiara Evans, MS\*, Armed Forces Medical Examiner System, Dover Air Force Base, DE; Joshua Seither, PhD, Armed Forces Medical Examiner System, Dover Air Force Base, DE; Jessica Knittel, MS, Armed Forces Medical Examiner System, Dover Air Force Base, DE; Erin Karschner, PhD, Armed Forces Medical Examiner System, Dover Air Force Base, DE; Jeff Walterscheid, PhD, Armed Forces Medical Examiner System, Dover Air Force Base, DE*

**Learning Overview:** After attending this presentation, attendees will be familiar with the steps needed to differentiate delta-8 and delta-9-Tetrahydrocannabinol (THC) from each other and other THC isomers. Attendees will also understand the importance of confirming the presence of delta-8 and delta-9-THC in forensic casework.

**Impact Statement:** This presentation impacts the forensic science community by raising awareness about the growing trend of detecting delta-8-THC, either with or without delta-9-THC, and how it can interfere with confirmation, reporting, and interpretation.

In the past few years, there has been increasing interest in expanded phytocannabinoids analysis. Previously, the presence of delta-9-THC and/or its metabolites in biological fluids indicated exposure to cannabis or cannabis products. More recently, cannabinoid analysis challenges involve the investigation of Cannabidiol (CBD) product usage, determination of THC and metabolite origins from cannabis or synthetic dronabinol, and separation of delta-8-THC, which can obfuscate delta-9-THC identification.

It is well-known that the acid catalyzed intramolecular cyclization of CBD can yield various proportions of delta-8-THC and delta-9-THC, which is influenced by the type of acid, solvent, temperature, and incubation time. Delta-8-THC is available to consumers in edible products, syrups, vaping oils, concentrates, and infused flour. A mixture of delta-8 and delta-9-THC and their carboxylated metabolites are often seen in routine laboratory analysis. If the analytical methods are not sufficient, these isomers may overlap and interfere with acceptable reporting criteria.

A Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed to resolve delta-8-THC, delta-9-THC, and their carboxylated metabolites. Unfortunately, at the time of development an 11-hydroxy-delta-8-THC reference standard was not available; therefore, it was not included in this analytical method. With consumer products now containing delta-6a,10a and delta-10-THC, it was important to characterize additional THC isomers that may interfere with the analytical method. Using the developed method, delta-6a,10a and delta-10-THC did not interfere with delta-8 and delta-9-THC, as these variants eluted outside the Multiple Reaction Monitoring (MRM) acquisition windows of the targeted analytes.

Since the beginning of 2021, delta-8 THC and/or delta-8-THCCOOH have been confirmed in over 25 human performance and postmortem cases. Without an adequate method of isomer separation, these cases would not be able to be reported to requesting authorities. After altering the chromatography parameters, improved peak resolution allowed for accurate determination of these two species in routine analysis. Authentic case examples presented will include delta-9-THC combined with low but reportable delta-8-THC, delta-8-THC with delta-9-THC absent or below reporting limits, and the more commonly encountered mix of roughly equal delta-8 and delta-9-THC results. It is important to note how these various situations may affect the acceptance criteria for reporting evidence of cannabinoid use.

This presentation will examine representative data and discuss solutions to THC isomer separation. If proper analytical techniques are not available, it is possible to overlook or misinterpret evidence of cannabinoid exposure. The United States military maintains a zero tolerance policy against using cannabis products to maintain safety, health, and mission readiness in the workforce. These cases underscore the need for vigilance when evaluating casework with proper confirmatory testing to reveal significant findings such as delta-8-THC and metabolites.

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### Delta-8-THC, Delta-9-THC, LC/MS/MS

## K35 Impaired Driving Cases With Clonazepam

*Nicholas B. Tiscione, BS\*, Palm Beach County Sheriff's Office, West Palm Beach, FL*

**Learning Overview:** After attending this presentation, attendees will increase their competence in the interpretation of, and need for, clonazepam testing in impaired driving investigations.

**Impact Statement:** This presentation will impact the forensic science community by outlining the increasing incidence, polypharmacy, and a case report of clonazepam in impaired driving investigations.

**Introduction:** Novel benzodiazepines have been increasing in seized drug submissions. Clonazepam has become one of the most frequently detected compounds in counterfeit alprazolam tablets. Clonazepam is a high-potency benzodiazepine that requires sensitive instrumentation and methods for detection in blood and urine specimens and may also require including the metabolite 8-Aminoclonazepam (8-AMC) to detect its use.<sup>1</sup> In 2019, 16 novel and non-routine benzodiazepines, including clonazepam, were added to the urine and blood Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) confirmation methods. In 2019 and 2020 only 9% and 8%, respectively, of immunoassay-positive benzodiazepine cases were not able to be confirmed. From January 1 to May 10, 2021, 24% of positive blood and urine specimens were not able to be confirmed. For cases from April 1 to May 10, 2021 that percentage was 37%, demonstrating a rapidly increasing trend. Revalidation was conducted to add 8-AMC to the scope of testing and decrease the quantitation range of clonazepam in blood from 5–100 to 1–20ng/mL.

**Methods:** For all driving under the influence of drugs blood cases, a volatile analysis was followed by an 11-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) and basic extraction with scan Gas Chromatography/Mass Spectrometry (GC/MS). Urine specimens were tested with a 9-panel ELISA and a basic extraction with scan GC/MS. All positive results were confirmed with GC/MS and/or LC/MS/MS, and/or LC with high-resolution MSMS. The ELISA cutoff for benzodiazepines using clonazepam as the target was 10ng/mL for blood and 25ng/mL for urine. The limit of quantitation for clonazepam and Limit Of Detection (LOD) for 8-AMC was 1.0ng/mL for blood by LC/MS/MS. The LOD for clonazepam and 8-AMC in hydrolyzed urine by LC/MS/MS was 20 and 100ng/mL, respectively.

**Results:** Since updating the blood (~1.5 months) and urine (~2.5 months) confirmation methods, 8-AMC was the most frequently identified benzodiazepine in blood and urine specimens submitted for impaired driving investigations. In urine, 8-AMC was identified in 14/44 cases (32%) and was second only to carboxy **Tetrahydrocannabinol** (carboxy-THC) (52%). Of note, the parent clonazepam was only identified in three urine specimens during the same period. In 26 blood cases tested since the method update, 8-AMC was identified in 10 (38%) with clonazepam identified in 8 (31%). For blood cases, 8-AMC was second only to fentanyl (50%) for the most commonly identified drug. Seven blood cases with quantitative results for clonazepam ranged from 2.9 to 19ng/mL.

Other drugs were identified in the vast majority of the clonazepam cases. In blood cases since 2019, 14 of the 15 positive cases (93%) also contained another drug. The most common combinations of other active drugs in the blood cases were opioids (87%), stimulants (60%), and other benzodiazepines (47%). In one blood case where 8-AMC was the only compound identified, officers observed impairment consistent with a Central Nervous System (CNS) depressant. The driver crashed into a line of cars stopped at a red light at a high rate of speed after being observed having difficulty maintaining his lane and driving too slowly on the roadway. The driver was observed to have problems maintaining balance, slow, thick slurred speech, and some difficulty following instructions.

**Discussion/Conclusion:** The incidence of clonazepam in impaired driving investigations has increased dramatically in 2020. In one blood case where 8-AMC was the only compound identified, impairment to driving ability and normal faculties consistent with a CNS depressant was observed. Detection of this potent benzodiazepine requires sensitive methods and/or inclusion of the metabolite 8-AMC. Other drugs were identified in the vast majority (93%) of the cases in this jurisdiction.

### Reference(s):

1. Krotulski, A.J.; Papsun, D.M.; Logan, B.K. (2020) Updated Trend Reporting for the NPS benzodiazepine Clonazepam Based on Data-Mining for 8-Aminoclonazepam. [https://www.npsdiscovery.org/wp-content/uploads/2020/11/Public-Alert\\_Clonazepam\\_NPS-Discovery\\_110520.pdf](https://www.npsdiscovery.org/wp-content/uploads/2020/11/Public-Alert_Clonazepam_NPS-Discovery_110520.pdf) (accessed May 20, 2021).

### Clonazepam, 8-Aminoclonazepam, DUID

## K36 Gabapentin in Driving Under the Influence of Drugs (DUID): An Update

Jolene Bierly, MSFS\*, NMS Labs, Horsham, PA; Ayako Chan-Hosokawa, MS, NMS Labs, Horsham, PA

**Learning Overview:** After attending this presentation, attendees will better understand the current prevalence and impact of gabapentin in DUID cases.

**Impact Statement:** This presentation will impact the forensic science community by detailing the current prevalence and impact of gabapentin in DUID cases.

**Introduction:** Gabapentin has been approved by the Food and Drug Administration (FDA) for adjunctive therapy in the treatment of epilepsy and neuralgia, but is increasingly being prescribed for multiple off-label uses, including insomnia, anxiety, migraine, and as an adjunct in pain management. Side effects include somnolence, dizziness, ataxia, nystagmus, and fatigue. There are previous reports of impaired driving attributed to gabapentin.<sup>1</sup> This group previously reported on the prevalence of gabapentin in DUID casework submitted to NMS Labs from 2015–2017. During that time, gabapentin testing was only available via a targeted Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) test. This work documents the changes in gabapentin prevalence in DUID cases since its inclusion in the Tier II DUID testing panel in 2020.

**Method:** Gabapentin was included in the laboratory's Tier II DUID expanded panel in 2020. The expanded panel includes a Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) screen plus an Enzyme-Linked Immuno-Sorbent Assay (ELISA) panel. Gabapentin is currently screened via the ELISA (Reporting Limit [RL] of 5.0mcg/mL) and confirmed via LC/MS/MS (RL 1.0mcg/mL). Antemortem blood specimens submitted for testing via the drugged driving test panel between January 2020 and June 2021 were reviewed. Cases where gabapentin testing was canceled for any reason were excluded.

**Results:** Between January 2020 and June 2021, there were 2,890 Tier II DUID expanded panels ordered. Gabapentin screened positive in 229 of these with an overall positivity rate of 7.9%; 219 cases confirmed gabapentin with a mean  $\pm$ Standard Deviation ( $\pm$ SD) and range of  $9.9 \pm 7.1$ mcg/mL (1.6–63mcg/mL). Polypharmacy was common with 95% of gabapentin cases confirming positive for at least one other drug. Common drug class combinations include gabapentin with opioids (54%), cannabinoids (42%), and stimulants (42%). There were 11 gabapentin-only cases between 2020 and March 2021. Mean  $\pm$ SD and range of gabapentin concentrations in those cases were  $10 \pm 7.2$ mcg/mL (2.4–28mcg/mL). A review of those cases revealed “erratic driving” was the most commonly reported driving behavior.

**Conclusion:** This review found an overall gabapentin positivity of 7.9% in DUID cases since January 2020. The mean and median concentrations of gabapentin were significantly higher than those reported in therapeutic use of trough plasma gabapentin concentrations of 2.6mg/L following a dose of 900mg/day, and an average concentration of 4.8mg/L in patients receiving 1,800mg/day, sampled randomly. Significant impairment to driving and human performance was observed in gabapentin-only cases. Approximately 95% of the cases in this review involved gabapentin in combination with other drugs.

### Reference(s):

<sup>1</sup> Peterson, B.L. Prevalence of gabapentin in impaired driving cases in Washington State in 2003-2007. *J Anal Toxicol.* 2009 Oct;33(8):545-9.

### Gabapentin, Drugged-Driving, Human Performance Toxicology

## K37 An Assessment of the National Safety Council's Tier 1 and Tier 2 Scope Recommendations in Authentic Driving Under the Influence of Drugs (DUID) Cases

Grace Cieri, BS\*, CFSRE, Willow Grove, PA; Amanda Mohr, MSFS, CFSRE, Willow Grove, PA; Melissa Fogarty, CFSRE, Willow Grove, PA; Barry Logan, PhD, NMS, Horsham, PA

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the prevalence of drugs detected in suspected DUID cases that are comprehensively testing and aligned with the scope recommendations for routinely detected and more esoteric drugs developed by National Safety Council's Alcohol, Drugs and Impairment Division Committee (NSC-ADID).

**Impact Statement:** This presentation will impact the forensic science community by providing an evaluation using comprehensive broad scope testing on authentic DUID cases, offering a highly comprehensive insight into the etiology of the problem in the United States. This information will benefit the criminal justice system by providing a more accurate picture of the drugs contributing to DUID, and the appropriate scope for contemporary drug testing scopes that can be used to update the NSC-ADID recommendations. This process will also provide better support for the drug recognition program and higher quality consolidated data for epidemiological studies and public policy.

Over the past decade, there has been an increase in the legalization of recreational drugs, use of Novel Psychoactive Substances (NPS), and prescription and over-the-counter drugs contributing to impaired driving becoming a larger problem and public health threat. Added to this problem is the scope of testing used by some laboratories, the practice of stop-limit testing, and the lack of standardization between laboratories. These factors have led to the under-reporting of drugs that are not routinely screened for as well as the not detecting emerging drugs such as NPS.

Discarded and deidentified blood samples submitted for analysis in suspected DUID cases were analyzed for the study. Two independent liquid-liquid extractions were performed to analyze for basic drugs and for synthetic cannabinoids. Borate buffer (1 mL, 0.1 M, pH 10.4) was added to blood (0.5 mL), followed by the addition of N-butyl chloride/ethyl acetate (70:30 v:v, 3 mL). For the synthetic cannabinoids and their metabolites, blood (0.5 mL) was basified with TRIS HCL (1.0 M, pH 10.2) and extracted with methyl tert-butyl ether (3 mL). Samples for both extractions were dried to completion and reconstituted in initial mobile phase (200 µL).

Both extracts were analyzed using a SCIEX™ TripleTOF® 5600+ Liquid Chromatography/quadrupole Time-Of-Flight (LC/qTOF) coupled with Shimadzu® Nexera Ultra High-Performance Liquid Chromatography (UHPLC). A reverse phase gradient of ammonium formate (1 mM, pH 3) and methanol/acetonitrile (50:50) with Phenomenex® Kinetex C18 analytical column (50 mm x 3.0 mm, 2.6 µm) was used on both sample types with the corresponding gradient for the basic and synthetic cannabinoid methods. The basic drug panel library contains over 900 drugs and metabolites, and the synthetic cannabinoid panel contains over 300 drugs and metabolites. Both libraries are updated regularly as new drugs are identified.

To date, 694 samples have been analyzed and processed on both the basic drug screening panel and the synthetic cannabinoid panel. The results were divided into three categories: Tier I, referring to drugs routinely tested for in laboratories; Tier II, compounds laboratories may or may not elect to include these in their screening process; and NPS, which is a subcategory under Tier II.

Results for Tier I included methamphetamine ( $n=108$ ; 15.6%) followed by amphetamine ( $n=107$ ; 15.4%), fentanyl ( $n=91$ ; 13.1%), cocaine ( $n=48$ ; 7.0%), and alprazolam ( $n=27$ ; 3.9%). Ethanol was found in 231 cases at an average concentration of 0.156 ( $\pm 0.07$ ) g/100 mL (median 0.152 g/100 mL) with a range of 0.11–0.610 g/mL. Delta-9-Tetrahydrocannabinol (THC) was found in 352 cases with an average concentration of 8.8 ( $\pm 10$ ) ng/mL (median 5.6 ng/mL). Tier II drugs were identified with less frequency. Diphenhydramine was identified in 35 cases, followed by cyclobenzaprine ( $n=24$ ; 3.5%), hydroxyzine ( $n=24$ ; 3.5%), and trazodone ( $n=20$ ; 2.9%). Results for NPS are 8-aminoclonazepam in 21 cases, followed by etizolam ( $n=11$ ; 1.6%) and fluorofentanyl ( $n=11$ ; 1.6%).

The findings from this evaluation suggest the most recent recommendations for NSC-ADID Tier I and Tier II drugs are appropriately aligned based on prevalence and frequency with which these drugs are detected in DUID casework samples. From the results to date, only the following compounds included in Tier I have not been detected: meprobamate, alpha-hydroxyalprazolam, and oxymorphone.

### Impaired Driving, Toxicology, Drugs

## K38 The Use of Statistical Models to Evaluate Signs of Cannabis Impairment During Drug Recognition Expert (DRE) Evaluations

Karen Woodall, PhD, University of Toronto, Mississauga, Ontario, Canada; Rachel Ram, BSc\*, University of Toronto, Mississauga, Ontario, Canada; Reed Holland, York Regional Police Service, Aurora, Ontario, Canada

**Learning Overview:** After attending this presentation, attendees will have learned which indicators used by DREs are significant for determining cannabis impairment using different statistical models.

**Impact Statement:** This presentation will impact the forensic community by providing statistical support for observations frequently used by DREs to identify cannabis-impaired drivers.

**Objective:** To evaluate suspected drug impaired driving cases where the DRE's observations were confirmed by toxicology analysis as cannabis-only, polydrug-cannabis, and cannabis-negative cases to identify which observations are associated with impairment resulting from recent cannabis use.

**Background:** The scientific merit of the DRE's is often questioned during legal proceedings due to limited scientific literature that demonstrates the DRE evaluation's sensitivity to cannabis impairment. Research evaluating DRE examinations found that officers were often accurate in identifying the drug category; however, the false negative rate for cannabis was higher than other drug classes.<sup>1</sup> Published literature evaluating the association between DRE metrics and cannabis impairment has compared the prevalence of these observations between individuals impaired by cannabis and non-impaired subjects and concluded that eyelid tremors, rebound dilation, dilated pupils, and elevated blood pressure are associated with cannabis impairment.<sup>2-4</sup> This study investigated data from impaired driving investigations where DRE observations and urine toxicology results were evaluated to see if statistical modeling could predict cannabis-positive and cannabis-negative cases.

**Methods:** One hundred ninety-one DRE evaluations and corresponding urine toxicology results were evaluated to create two statistical models: a binary model, which distinguishes between cannabis-positive and cannabis-negative cases; and a multinomial model, which distinguishes between cannabis-positive, polydrug-cannabis, and cannabis-negative. Decision trees and logistic regression were used to create the statistical models, resulting in four models: (1) binary decision tree; (2) multinomial decision tree; (3) binary logistic regression; and (4) multinomial logistic regression. Cross-validation was conducted to assess the accuracy and performance of each model.

**Results:** The binary decision tree classified cannabis-positive and cannabis-negative cases with 81.68% accuracy using three variables: first using eyelid tremors, then muscle tone, and rebound dilatation. The presence of eyelid tremors and rebound dilation were good predictors of cannabis-positive cases. The multinomial decision tree classified cannabis-only, polydrug-cannabis, and cannabis-negative cases with 70.16% accuracy using three variables: first with eyelid tremors, rebound dilation, and number of cues observed during the Walk And Turn (WAT) test. In the presence of eyelid tremors, <2 cues WAT predicted cannabis-only cases whereas  $\geq 2$  cues predicted polydrug-cannabis cases. Binomial logistic regression (81.48% accuracy) differentiated positive and negative cases using eyelid tremors ( $p < 0.001$ ), lack of convergence ( $p < 0.001$ ), rebound dilation ( $p < 0.001$ ), Horizontal Gaze Nystagmus (HGN) ( $p < 0.01$ ), and muscle tone ( $p < 0.05$ ). Eyelid tremors, lack of convergence, rebound dilation, and normal muscle tone increased the odds of a case being cannabis-positive, whereas presence of HGN and rigid muscle tone increased the odds of a case being cannabis-negative. The multinomial regression model was 70.37% accurate and did not produce statistically significant indicators to distinguish between cannabis-only and polydrug-cannabis cases.

**Conclusion:** The results from this study provides statistical evidence that eyelid tremors and rebound dilation are the most robust observations that DRE's use when determining if there was recent cannabis use regardless of if the subject was impaired by cannabis alone or a combination with other drugs.

### Reference(s):

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2. Hartman, R.L.; Richman, J.E.; Hayes, C.E.; Huestis, M.A. (2016). Drug Recognition Expert (DRE) examination characteristics of cannabis impairment. *Accident Analysis & Prevention*, 92, 219-229.
3. Declues, K.; Perez, S.; Figueroa, A. (2016). A 2-Year Study of  $\Delta 9$ -tetrahydrocannabinol Concentrations in Drivers: Examining Driving and Field Sobriety Test Performance. *Journal of forensic sciences*, 61(6), 1664-1670. <https://doi.org/10.1111/1556-4029.13168>.
4. Declues, K.; Perez, S.; Figueroa, A. (2018). A Two-Year Study of  $\Delta 9$  Tetrahydrocannabinol Concentrations in Drivers; Part 2: Physiological Signs on Drug Recognition Expert (DRE) and non-DRE Examinations. *Journal of forensic sciences*, 63(2), 583-587.

### Cannabis, Drug Recognition Evaluation, Impairment

## K39 Evaluating Cannabinoid Interference and Drug Stability in Oral Fluid for Driving Under the Influence of Drugs (DUID) Testing

Jasmine Maxwell, MSFS\*, University of Alabama at Birmingham, Birmingham, AL; Elizabeth Gardner, PhD, University of Alabama at Birmingham, Birmingham, AL; Curt E. Harper, PhD, University of Alabama at Birmingham, Birmingham, AL and Alabama Department of Forensic Sciences, Hoover, AL

**Learning Overview:** After attending this presentation, attendees will have a better understanding of potential novel cannabinoid interference and the stability of cannabinoids in Oral Fluid (OF).

**Impact Statement:** This presentation will impact the forensic science community by presenting data on analyte and matrix interference on a cannabinoid OF assay with novel cannabinoids. This presentation will provide knowledge to the forensic science community about cannabinoid stability in authentic OF case specimens.

The Scientific Working Group for Forensic Toxicology (SWGTOX) method validation guidelines provide instructions on how to evaluate interference and stability. It is important that laboratories continuously monitor method performance, potential new interferents (e.g., isomers, metabolites), and analyte stability. The collection device, buffer, analyte chemical properties, and storage conditions are all factors in the stability of drug concentrations in OF.

OF drug testing is efficient due to its easy, fast, minimally invasive, gender-neutral collection. The applications of drug testing originated in workplace, probation, and pain management facilities, but now include use in DUID and postmortem investigations. OF can be used to establish probable cause at the roadside using devices such as the Dräger DT5000 or the Abbott® SoToxa and for laboratory evidentiary confirmation in DUID cases. Alabama has recently passed a new law, SB258, which gives OF tests the same “implied consent” legal standing as breath and blood test to detect alcohol and drugs after an arrest.

A previously validated liquid-liquid extraction for delta-9-Tetrahydrocannabinol ( $\Delta^9$ -THC), 11-Hydroxy-delta-9-Tetrahydrocannabinol ( $\Delta^9$ -THC-OH), delta-9-Tetrahydrocannabinol Carboxylic acid ( $\Delta^9$ -THC-COOH), Cannabidiol (CBD), Cannabinol (CBN), and Cannabigerol (CBG) followed by analysis using an Agilent® 6460 Triple Quadrupole Tandem Mass Spectrometer was used for this study. OF samples were collected using Quantisal™ collection devices from DUI subjects. Novel cannabinoids (6aR,9S)- $\Delta^{10}$ -tetrahydrocannabinol, (6aR,9R)- $\Delta^{10}$ -tetrahydrocannabinol, 9R- $\Delta^6$ a,10a-tetrahydrocannabinol, 9S- $\Delta^6$ a,10a-tetrahydrocannabinol, delta-8-Tetrahydrocannabinol ( $\Delta^8$ -THC), delta-8-Tetrahydrocannabinol Carboxylic acid ( $\Delta^8$ -THC-COOH), and 11-Hydroxy-delta-8-Tetrahydrocannabinol ( $\Delta^8$ -THC-OH) were evaluated for target interference with previously validated analytes listed above. Suspected negative OF specimens from volunteer subjects were evaluated for matrix interferences. Previously analyzed and simulated casework specimens were evaluated for stability at various time points up to two years.

Mobile phase A was 5mM of ammonium formate with 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. An Agilent® Poroshell 120 EC-C18 column (2.1 x100mm, 2.7 micron) was used. The LC gradient originally started with 50% mobile phase B for 1 minute, increasing to 65% B at 5 minutes, increasing to 95% B at 8 minutes, and holding at 95% B for 2 minutes. The modified method begins with 70% mobile phase B for 1 minute, increasing to 80% B at 4 minutes, and increasing to 99% B at 10 minutes, which resulted in baseline resolution of  $\Delta^9$ -THC,  $\Delta^8$ -THC, and (6aR,9R)- $\Delta^{10}$ -THC. Fourteen expectorate samples and one Quantisal™ sample had no interference in the blank matrix study. Thirty-two previously analyzed authentic specimens, collected from subjects in drug rehabilitation in Jacksonville, FL, were re-analyzed after two years ( $\pm$  two weeks) of storage at 4°C. All cases were previously positive for  $\Delta^9$ -THC. Upon reanalysis, 100% of cases were still positive at comparable concentrations. Preliminary analyses suggest effective stability of the Quantisal™ buffer over that time period.

Laboratories should evaluate their current methodology for potential interferents from novel cannabinoids, specifically  $\Delta^9$ -THC isomers and their metabolites. With the surge in  $\Delta^8$ -THC products throughout the United States, it is imperative that toxicological analyses have specificity to detect  $\Delta^8$ -THC and its metabolites. OF cannabinoid stability should also be evaluated over the expected testing time frame in the laboratory. This study has highlighted a cannabinoid OF method with suitable stability in Quantisal™ buffer and lack of interference.

### Reference(s):

1. Scientific Working Group for Forensic Toxicology. (2013). Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. *Journal of Analytical Toxicology*, 37(7), 452-474. 10.1093/jat/bkt054

### Oral Fluid, Cannabinoids, Interference



## K40 The Development and Validation of an Analytical Method for Ten Novel Designer Benzodiazepines in Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will learn a practical LC/MS/MS-based analytical method for selected designer benzodiazepines.

**Impact Statement:** This presentation will impact the forensic science community by providing information on an analytical method for selected designer benzodiazepines.

**Background/Introduction:** Designer benzodiazepines have become a rapidly growing group of novel psychoactive substances abused in the U.S.<sup>1</sup> They have created significant challenges for forensic toxicology laboratories in detection and confirmatory testing. In addition, they have created loopholes in regulation and legal proceedings due to the unestablished Drug Enforcement Administration (DEA) classification status. Emerging illicit production and use of designer benzodiazepines have drawn increasing interest and attention from the forensic toxicology community.

**Objective:** The purpose of this research was to develop and validate an analytical method to reliably detect and quantify prevalent designer benzodiazepines in human blood using LC/MS/MS. The analytes were selected based upon recent designer benzodiazepine prevalence in the United States and cross-reactivity with the benzodiazepine Enzyme-Linked Immuno-Sorbent Assay (ELISA) currently used in this study's laboratory.<sup>1</sup>

**Method:** The method was validated in accordance to the Academy Standards Board (ASB) Standard 036 (2019). The quantitative testing panel includes  $\alpha$ -hydroxyetizolam, clonazolam, flualprazolam, deschloroetizolam, flubromazolam, desalkylflurazepam, etizolam, and declazepam. Adinazolam and flubromazepam are for qualitative analysis.

Following addition of internal standards (etizolam-d3 and temazepam-d5) in the specimen, analytes were extracted by a solid phase extraction utilizing CEREX<sup>®</sup> CLIN II column. Analytes were separated with an Agilent<sup>®</sup> 1290 Infinity II LC equipped with a 2.1x50mm, 2.7 $\mu$ m C18 column. Detection was performed using an Agilent<sup>®</sup> 6470B QQQ system with Electrospray Ionization (ESI) in positive mode and dynamic Multiple Reaction Monitoring (MRM).

**Results:** All analytes showed high ELISA cross-reactivity (135%–607%) at 20 g/mL. Calibration curves for quantitative analysis achieved R<sup>2</sup> values >0.99. LC/MS/MS run time was 5.5 minutes with analytes eluting between 2.1 and 3.5 minutes. Limits of Detection/Limits Of Quantitation (LODs/LOQs) for all ten analytes were 0.5ng/mL. A dynamic range of 0.5 to 100ng/mL was established for quantitation. All eight analytes for quantitative analysis showed acceptable %bias and %CV within  $\pm$ 20% at 1.5, 10, and 80ng/mL. No significant carryover was observed following samples containing the analyte up to 250ng/mL for all ten analytes. Dilution integrity was challenged with dilution factors of 2, 5, and 10. Average quantitation %bias was <20% with each dilution factor for all eight analytes. Matrix interference and ionization suppression/enhancement from multiple blank human blood samples and exogenous substances were evaluated. No significant interference was observed, except for deschloroetizolam coeluting with alprazolam. Both punctured and unpunctured samples stored inside LC/MS/MS multi-sampler at 4°C showed acceptable stability for at least 72 hours.

**Conclusion/Discussion/Future Applications:** The overall results demonstrated a reliable analysis of several emerging designer benzodiazepines in human blood. Once the method is approved for casework, the laboratory plans to apply the method to case samples that previously screened positive by ELISA but confirmed negative for traditional benzodiazepines. The development of the method for urine specimens is currently in progress.

### Reference(s):

- <sup>1</sup> *Recommended Scope for NPS Testing in the United States Q2 2021*. [https://www.npsdiscovery.org/wp-content/uploads/2021/07/Q2-2021-NPS-Scope-Recommendations\\_NPS-Discovery\\_072821.pdf](https://www.npsdiscovery.org/wp-content/uploads/2021/07/Q2-2021-NPS-Scope-Recommendations_NPS-Discovery_072821.pdf).

### Designer Benzodiazepines, LC/MS/MS, Drug Testing

## **K41 Quantitation of Synthetic Cannabinoid, 5F-MDMB-PICA, and its Metabolites in Authentic Human Biological Samples using Liquid Chromatography Tandem Quadrupole Mass Spectrometry (LC-QQQ-MS)**

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**Learning Overview:** After attending this presentation, attendees will be able to describe the method for quantitating the synthetic cannabinoid 5F-MDMB-PICA and assess concentrations in human samples.

**Impact Statement:** This presentation will impact the forensic toxicology community by providing methodology for the quantitation of a prevalent synthetic cannabinoid, 5F-MDMB-PICA, and showcasing authentic quantitative human data for this synthetic cannabinoid and its metabolites.

Synthetic cannabinoids, also called “K2” or “Spice,” are Novel Psychoactive Substances (NPS) and these drugs are constantly evolving. Varying chemistries and structures of this class and their recreational use provide challenges for forensic toxicologists. 5F-MDMB-PICA is a potent CB<sub>1</sub> receptor agonist first detected in Europe in 2016. This drug has subsequently been identified in the drug supply in the United States throughout 2021, at times being the most prevalent synthetic cannabinoid detected. As the popularity of synthetic cannabinoids increases, the need for sensitive methodology to determine concentrations in human biological samples is of great importance to assess the degree of exposure.

A sample preparation and extraction procedure were developed and validated. The method included 5F-MDMB-PICA and several of its metabolites (5F-MDMB-PICA 3,3-dimethylbutanoic acid, 5OH-MDMB-PICA, 6'-OH-5F-MDMB-PICA, 2COOH-MDMB-PICA, and 4OH-5F-MDMB-PICA 3,3-dimethylbutanoic acid). 5F-MDMB-PICA-D5 and 5F-MDMB-PICA 3,3-dimethylbutanoic acid-D5 were used as internal standards. The calibration range for 5F-MDMB-PICA was 0.5 to 500ng/mL, and the calibration range for the metabolites was 1 to 500ng/mL. Samples (0.2mL) were prepared using an acidic liquid-liquid extraction with 5% phosphoric acid in water and an extraction solvent consisting of hexane, ethyl acetate, and MTBE (80:10:10 v:v:v).

Quantitation was performed using a Waters® Xevo TQ-S micro LC/QqQ/MS. Chromatographic separation was achieved on an Agilent® InfinityLab Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7µm) under gradient elution. Mobile phase compositions were 0.1% formic acid in water and 0.1% formic acid in methanol. The flow rate was 0.4mL/min, injection volume was 5µL, and column temperature was 30°C. Authentic samples suspected of containing 5F-MDMB-PICA were obtained for analysis from NMS Labs. Samples were collected as part of for-cause forensic investigations and were deidentified prior to use; however, demographic data were still available.

Quantitative analysis was performed on blood (*n*=44), serum/plasma (*n*=2), urine (*n*=7), vitreous fluid (*n*=10), bile (*n*=1), and gastric contents (*n*=1). Samples were collected between 2018 and 2019. The case types were primarily postmortem, but Driving Under the Influence of Drugs (DUID) and clinical cases were also included. Cases originated from 12 states. 5F-MDMB-PICA was qualitatively identified in 17 blood samples and quantitatively confirmed in 23 blood samples. Blood concentrations of 5F-MDMB-PICA ranged from 0.5 to 6.2ng/mL (mean: 2.2 ± 1.7ng/mL, median: 1.4ng/mL). All DUID cases (*n*=4) had concentrations between 0.5 and 1ng/mL.

5F-MDMB-PICA 3,3-dimethylbutanoic acid was confirmed in 44 blood samples ranging from 1.7 to 272ng/mL (mean: 39 ± 61ng/mL, median: 17ng/mL). 5OH-MDMB-PICA (*n*=23, mean: 6.4 ± 9.2ng/mL, median: 3.5ng/mL, range: 1–46ng/mL), 4'-OH-5F-MDMB-PICA 3,3-dimethylbutanoic acid (*n*=24), and 2COOH-MDMB-PICA (*n*=1) were also identified in the blood samples.

5F-MDMB-PICA was also confirmed in urine (*n*=6), serum/plasma (*n*=2), vitreous fluid (*n*=5), bile (*n*=1), and gastric contents (*n*=1, 36ng/mL). 5F-MDMB-PICA was discovered alongside other synthetic cannabinoids (4F-MDMB-BINACA), classic cannabinoids (THC), benzodiazepines, stimulants, and therapeutic drugs.

5F-MDMB-PICA is a prevalent synthetic cannabinoid and was confirmed at low ng/mL concentrations. Two primary metabolites, 5F-MDMB-PICA 3,3-dimethylbutanoic acid and 5OH-MDMB-PICA, were also identified and show the need for sensitive instrumentation and a wide calibration range. 5F-MDMB-PICA is still being identified in 2021, and the presence of 5F-MDMB-PICA with other synthetic cannabinoids, such as 4F-MDMB-BINACA, shows the need for continued research on these newly emerging drugs.

### **Toxicology, Human, Overdose**

## K42 An Explanation and the Impacts of a Class-Wide Ban on Synthetic Cannabinoids: What Does the Future Hold for This Already Challenging Novel Psychoactive Substances (NPS) Subclass?

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**Learning Overview:** After attending this presentation, attendees will be able to assess the impacts of a class-wide ban on synthetic cannabinoids and how this could affect forensic casework and testing.

**Impact Statement:** This presentation will impact the forensic science community by expanding knowledge of synthetic cannabinoids as it relates to legislation, nomenclature, and emerging drug trends.

Synthetic cannabinoids make up the most structurally diverse subclass of NPS and, as such, are undoubtedly the most challenging analytical targets for forensic scientists. Analytical challenges arise due to chemical diversity compared to more traditional drugs and NPS subclasses, compounded by the need for high instrumental sensitivity due to increasing potencies. Reporting and interpretation are also challenging due to inconsistent naming conventions and lack of published data regarding pharmacology, toxicity, and drug concentrations. Remaining abreast of current knowledge for synthetic cannabinoids requires constantly evolving expertise.

Through the NPS Discovery program, the non-profit Center for Forensic Science Research and Education (CFSRE) tracks the emergence of new synthetic cannabinoids through both conventional and non-conventional data sources. Since 2018, this laboratory has employed aggressive sampling and data-mining workflows via Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (L/-qTOF/MS) to discover new synthetic cannabinoids on the recreational drug market and to monitor trends with respect to drug positivity and proliferation. The largest source of data remains the analysis of authentic forensic toxicology samples, followed by drug materials and clinical samples. In addition, this study's scientists review drug use forums and online gray market vendor websites to gather intelligence about next-generation NPS.

In May 2021, China announced new national legislation that included the class-wide scheduling of synthetic cannabinoids based on core structural features—a move that surprised most in forensic science circles. While core-structure scheduling is not a new concept (e.g., fentanyl and its analogs), this announcement differed in that its purpose was to control an entire subclass of drugs rather than an individual structurally related family. Synthetic cannabinoids were structurally outlined under the regulation based on combined core/linker moieties and further defined by head and tail constituents. Core/linker structures included commonly encountered indoles and indazoles, as well as gamma-carboline, cyclohexylphenols, and others. Tail constituents included varying length hydrocarbons, both substituted and unsubstituted, as well as heterocyclic groups. Head constituents included a large variety of substituted and unsubstituted groups. The effective date for this legislation was listed as July 2021.

In Q1 and Q2 2021, MDMB-4en-PINACA, 5F-MDMB-PICA, ADB-BINACA, and 4F-MDMB-BICA were the most prevalent synthetic cannabinoids identified in forensic casework in the United States. Of these four, 5F-MDMB-PICA was the only drug to be explicitly scheduled by the Drug Enforcement Administration as of August 2021. However, all four drugs are covered under the new Chinese ban: MDMB-4en-PINACA and ADB-BINACA are indazole derivatives while 5F-MDMB-PICA and 4F-MDMB-BICA are indole derivatives. By June 2021, this study's laboratory had already identified a synthetic cannabinoid that would not be covered by the ban: 5F-AB-PFUPPYCA. In the next few months, online reviews of gray market vendors showed the gradual disappearance of variants that were included under the outlined legislation and the emergence of those that could be considered “legal,” including a series of MDA-19 (BZO-HEXOXIZID) analogs. In August 2021, tentative identifications of the pentyl and 5-fluoropentyl analogs of MDA-19 were reported (or BZO-POXIZID and 5F-BZO-POXIZID by systematic nomenclature, respectively).

National and international drug control policies can impact the presence and prevalence of NPS on recreational drug markets. Due to recent actions, the synthetic cannabinoid subclass will require increased monitoring over the coming months as new drugs emerge with the goal of evading class-wide bans. Forensic toxicologist must continue research and evaluation of these substances, especially as pharmacology and toxicity are unknown.

### Synthetic Cannabinoid, Legislation, Toxicology

## K43 The 2021 Novel Psychoactive Substance (NPS) Landscape: The New, the Nuanced, and the Long-Haulers

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**Learning Overview:** After attending this presentation, attendees will be able to describe the 2021 NPS landscape encountered in forensic toxicology casework.

**Impact Statement:** This presentation will impact the forensic sciences by providing an up-to-date snapshot of emerging substances.

NPS are generally characterized as those that have emerged on recreational drug markets since the mid-2000s, whether newly synthesized or repurposed from past scientific research, and/or substances that are being used in a differing manner from original intended use. The NPS landscape is in a constant state of flux as new substances enter the market and old, newly controlled, and/or those out-of-favor exit. These challenges require substantial resources in monitoring and investigating new substances, implementing new or modified testing protocols, and disseminating information.

To provide a comprehensive and current snapshot of the 2021 NPS landscape, all blood cases reporting synthetic stimulants/hallucinogens, Synthetic Cannabinoids (SC), Designer Benzodiazepines (DBZD) and/or Novel Synthetic Opioids (NSO) for the first seven months of 2021 were queried from a large reference laboratory. Reports with more than five cases are listed below.

Synthetic stimulants, along with SCs, historically were the two main classes of NPS, with several generations of substances emerging since the mid-2000s. Eutylone continues to be the current dominant synthetic stimulant, accounting for over 95% of the synthetic stimulant/dissociative blood reports. For SCs, 5-fluoro-MDMB-PICA accounted for 48.2% of reported blood cases. MDMB-4en-PINACA and 4-fluoro-MDMB-BINACA accounted for 32 and 8.9%, respectively.

There has been an increase in number and variety of DBZD. In total, 15 different DBZD (with 3 metabolite pairs) have been reported in the first seven months of 2021. Etizolam, which was first reported to the European Union (EU) Early Warning System in 2011, accounted for over 62% of blood DBZD cases, along with its metabolite alpha-hydroxyetizolam. Flualprazolam, flubromazolam, clonazolam/8-aminoclonazolam, and bromazolam account for 15.9, 9.4, 6.0, and 2.9% of DBZD blood detections, respectively. Diclazepam/delorazepam, flubromazepam, and bromazepam account for 1.2, 0.9, and 0.7%, respectively.

NSOs, due to their significant threat to public health, have garnered a significant amount of attention since 2016. Despite core structure scheduling enacted for fentanyl analogs, many of these substances continue to be detected, although on an infrequent basis. Butyrylfentanyl, valerylfentanyl, and para-fluoroisobutyrylfentanyl were reported less than 13 times each, while a number of other fentanyl analogs were reported in lower numbers. Carfentanil continues to maintain a presence, accounting for 8.3% of reported blood cases involving NSOs. Novel non-fentanyl related opioids isotonitazene, brorphine, and metonitazene have accounted for 0.5, 2.0, and 3.8% reported blood NSO cases. Accounting for over 82% of reported NSO findings, the frequency of para-fluorofentanyl detections and reporting has greatly increased in 2021. In most cases, fentanyl was detected in conjunction with para-fluorofentanyl, which raises the questions of sourcing, adulteration, and trafficking.

Other atypical opioid agonists include tianeptine and mitragynine; tianeptine has been reported in 12 blood cases and mitragynine has been reported over 1,060 times in the first seven months of 2021.

NPS reports in 2021 included over 40 compounds representing the various subclasses. Some of these compounds, including etizolam and mitragynine, have been reported for almost a decade, which begs the question of when to transition a compound from “emergent” and “NPS” to part of the routine or traditional drug testing scopes. Conversely, some NSOs are emerging and disappearing quickly, within lifespans of less than a year. Para-fluorofentanyl has emerged and proliferated so rapidly that its positivity characteristics are more aligned with supply chain for illicit narcotics rather than NPS. Assessment of the 2021 NPS landscape remains challenging. Understanding that positivity is underestimated due to lags in testing implementation and lack of inclusion in testing scopes is vital.

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### Novel Psychoactive Substances, Novel Synthetic Opioids, Designer Benzodiazepines

## K44 Multimodality Surveillance of Adverse Events of Designer Stimulants From 2013 to 2020

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**Learning Overview:** After attending this presentation, attendees will understand and be able to describe the adverse events and toxicological signs and symptoms associated with designer stimulant use.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of adverse events associated with designer stimulant use to better characterize international emerging drug threats.

**Introduction:** An important role of modern forensic and clinical toxicologists is to monitor the adverse events of Novel Psychoactive Substances (NPS). This presentation describes adverse events from case reports and reference data on NPS designer stimulants during an eight-year period from 2013–2020. The primary objective of this study is to assist in the assessment and interpretation of these cases as well as provide references for confirmation methods. Chemistry, pharmacology, adverse events, and user profiles (e.g., polypharmacy) for novel stimulants are provided including case history, clinical symptoms, autopsy findings, and analytical results.

**Methods:** Literature reviews were performed in PubMed® and Google® Scholar for publications dated between January 2017 and December 2020.<sup>1,4</sup> Search terms included NPS-specific names, general terms (e.g., “designer drugs,” “novel psychoactive substances”) and drug class (e.g., “designer stimulants”), as well as outcome-based terms (e.g., “overdose,” “death”). Government and website drug surveillance databases were also reviewed (e.g., National Forensic Laboratory Information System [NFLIS], European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], NPS Discovery). Abstracts published by the Society of Forensic Toxicologists (SOFT) and American Academy of Forensic Sciences (AAFS) were also investigated. Toxicological data and detailed case information were extracted, tabulated, analyzed, and organized by drug category. Similar methods were used in the initial study from 2013–2016.<sup>3</sup>

**Results:** From 2017 to 2020, This study found 64 case reports of designer stimulants reported in the literature and in drug surveillance systems for fatalities (41 cases), clinical treatment and hospitalization (21 cases), and Driving Under the Influence of Drugs (DUID) (2 cases). This is nearly a 400% increase in designer stimulants case reports compared to those reported in the previous four years.<sup>3</sup> Confirmed adverse events with associated toxidromes of ten designer stimulants were reported: 4F-MPH, 4-MEAP, 4F- $\alpha$ PVP, Dibutylone, EPH, MPPH, NEH, NEP,  $\alpha$ -EAP, and  $\alpha$ -PHP5. The toxicity profile of the designer stimulants included agitation, psychosis, delusions, aggression, irritability, paranoia, delirium, hallucinations, sedation, coma, abnormal behavior, altered fluctuating consciousness, tachycardia, hypertension, palpitations, increased respiration rate, hypothermia, mydriasis, rhabdomyolysis, compartment syndrome, and sweating. Common autopsy findings included organ edema and congestion. Almost 70% of the cases had multiple drugs confirmed. The observations and analytical methods associated with these postmortem, clinical, and DUID cases will be discussed.

**Conclusion:** This study provides insight and context of case findings for postmortem, clinical, and DUID reports for novel stimulants described in the literature and in digital government surveillance databases and websites during the past eight years.

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4. Mohr, L.A.; Logan, B.K.; Fogarty, M.F.; Krotulski, A.J.; Papsun, D.M.; Kacinko, S.L.; Huestis, M.A.; Roper-Miller, J.D. Reports of Adverse Events Associated with Use of Novel Psychoactive Substances, 2017–2020: A Review. Submitted to *J. Anal. Toxicol.*

### Novel Psychoactive Substances, Designer Stimulants, Adverse Events

## K45 An Evaluation of Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the Detection of Novel Psychoactive Benzodiazepines

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**Learning Overview:** This presentation will provide attendees with an understanding of the capability of ELISA to detect novel psychoactive benzodiazepines and select metabolites in toxicology casework.

**Impact Statement:** This presentation will impact the forensic science community by providing valuable insight into the use of ELISA technology to detect novel psychoactive benzodiazepines and metabolites in blood, thereby assisting in the interpretation of screening results to direct further testing.

With the continued emergence of novel psychoactive substances (NPS), the detection and confirmation of NPS has become of increasing interest to the forensic toxicology community. Several benzodiazepines are among the emerging group of NPS and, in response, an in-house Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed to detect 40 benzodiazepines. Among these are 17 NPS benzodiazepines that are not currently listed as having been investigated for cross-reactivity with the Immunalysis™ Benzodiazepine Direct ELISA kit.<sup>1,2</sup> The 17 benzodiazepines and/or metabolites are as follows: alpha-hydroxymidazolam, alpha-hydroxytriazolam, bromazolam, N-desmethyloclobazam, 3-hydroxyflunitrazepam, phenazepam, deschloroetizolam, clonazolam, delorazepam, diclazepam, flualprazolam, flunitrazolam, 4-hydroxyalprazolam, meclonazepam, methylclonazepam, nitrazolam, and pyrazolam.

In the study presented, the 17 compounds listed above were spiked individually into drug-negative blood (synthetic) at the corresponding Lower Limit of Quantitation (LLOQ) detailed in the LC/MS/MS method.<sup>1</sup> Each sample was then analyzed using the Tecan Freedom EVO 100/4 (with Tecan Sunrise Reader) to determine the cross-reactivity with the Benzodiazepine Direct ELISA kit. A specimen absorbance less than or equal to the cutoff calibrator absorbance indicates a positive finding, whereas a specimen absorbance that is higher than the cutoff calibrator but within 30% is considered elevated and an absorbance greater than the cutoff calibrator absorbance indicates negative.

The following compounds were positive at the LLOQ concentration: alpha-hydroxymidazolam (10ng/mL), alpha-hydroxytriazolam (10ng/mL), bromazolam (10ng/mL), N-desmethyloclobazam (10ng/mL), phenazepam (10ng/mL), delorazepam (5ng/mL), diclazepam (5ng/mL), flualprazolam (5ng/mL), flunitrazolam (5ng/mL), 4-hydroxyalprazolam (5ng/mL), methylclonazepam (5ng/mL), nitrazolam (5ng/mL), and pyrazolam (5ng/mL). Those compounds that did not flag as positive at the LOQ were then ran at higher concentrations until a positive result was obtained. The following four compounds were positive at higher concentrations: 3-hydroxyflunitrazepam (20ng/mL), deschloroetizolam (4ng/mL), clonazolam (10ng/mL), and meclonazepam (25ng/mL). The concentrations triggering a positive response is a particularly valuable demonstration considering a 100% ELISA positive corresponds to 100ng/mL of the target compound, oxazepam.

This study helps in understanding the cross-reactivity of the 17 benzodiazepines and/or metabolites studied and can help navigate testing protocol for specimens when novel psychoactive benzodiazepines are suspected.

### Reference(s):

1. Sofalvi, S.; Lavins, E.S.; Kaspar, C.L.; Michel, H.M.; Mitchell-Mata, C.L.; Huestis, M.A.; Apollonio, L.G. Development and Validation of an LC-MS-MS Method for the Detection of 40 Benzodiazepines and Three Z-Drugs in Blood and Urine by Solid-Phase Extraction. *Journal of Analytical Toxicology*. 2020 Oct 12; 44(7): 708-717.
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### ELISA, Benzodiazepines, Novel

## K46 An Analysis of Methylphenidate, Ethylphenidate, Lisdexamfetamine, and Amphetamine in Oral Fluid by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will understand how to effectively isolate and quantify various stimulants from Oral Fluid (OF).

**Impact Statement:** This presentation will impact the forensic community by providing a Solid Phase Extraction (SPE) method for cognitive-stimulants from OF. Additionally, a novel approach to quantify these stimulants using LC/MS/MS will be presented.

Attention-Deficit/Hyperactivity Disorder (ADHD) is a disorder in the brain that is more commonly studied in children and adolescents. In more recent years, there has been an increase in cognitive stimulant abuse at the collegiate level. Students illicitly use these medications to speed up brain activity to perform better in their studies. There are various medications that are commonly prescribed to treat ADHD such as Methylphenidate (MPH), Lisdexamfetamine (LDX), and Amphetamine (AMP). There are limited methods that analyze multiple compounds in a single assay using Solid Phase Extraction (SPE) and LC/MS. For this reason, the goal was to develop a broad method to isolate multiple cognitive stimulant drugs from OF. OF is an alternative matrix that is non-invasive and allows for quick collection. The objective of this study was to develop and validate a method to isolate MPH and its metabolite, Ethylphenidate (EPH), LDX and AMP and quantify them in OF utilizing LC/MS/MS.

MPH, EPH, LDX, and AMP were extracted using SPE. OF samples (250 $\mu$ L OF: 750 $\mu$ L Quantisal<sup>®</sup> extraction buffer) were fortified with calibrator (25 $\mu$ L) or control (10 $\mu$ L) and internal standard (25 $\mu$ L) solutions. Drugs were isolated using a pre-conditioned, mixed mode polymeric SPE column and eluted with 5% ammonium hydroxide in dichloromethane:isopropyl alcohol (80:20). Analysis was performed on an Agilent<sup>®</sup> 1290 Infinity liquid chromatograph coupled to an Agilent<sup>®</sup> 6470 Triple Quadrupole mass spectrometer with a gradient elution starting at 90:10 mobile phase A:B at 0.4mL/min. Mobile phase A was 5mM ammonium formate and 0.05% formic acid (FA) in diH<sub>2</sub>O. Mobile phase B was 0.1% FA in acetonitrile. A multiple reaction monitoring (MRM) method with one transition for qualification and one transition for quantification was used for analyte detection. This method was validated according to American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036. For proof of applicability, authentic OF samples ( $n=4$ ) were collected via Quantisal<sup>®</sup> OF collection device in an Institutional Review Board (IRB) -approved study and analyzed as above.

MPH, EPH, and AMP had a linear range of 0.5–500ng/mL and  $\sim$ 500ng/mL for LDX ( $R^2 > 0.99$ ). Limit of Detection (LOD) and Limit Of Quantitation (LOQ) for MPH, EPH, and AMP was 0.25 and 0.5ng/mL, respectively. LOD and LOQ for LDX were both 5ng/mL. Extraction recovery was  $>90\%$  for all analytes. Matrix effects displayed suppression for all analytes except LDX, which displayed enhancement. Bias ranged from  $-1$ – $6.1\%$  with maximum within-run precision of 15.7% for all analytes at three concentrations. Processed sample stability in the autosampler (4 $^{\circ}$ C, 24h) was evaluated and all compounds were considered stable ( $\pm 17\%$  bias). Dilution integrity was sustained at a factor of 1/10 ( $< 7.3\%$  bias). No carryover or interferences (exogenous, endogenous, or stable-isotope) were observed. Authentic OF samples were collected within six hours of prescribed dose and were positive for LDX ( $n=1$ ) and AMP ( $n=4$ ). LDX was detected at 5.8ng/mL, while mean (range) AMP concentrations were 34.4 (6.0–78.8) ng/mL. These results are consistent with the prescribed medications reported.

The developed technique provides a fully validated quantification method for select cognitive stimulants in OF by LC/MS/MS. Additionally, this method was used to successfully analyze authentic OF samples containing LDX and AMP. The present research is relevant for advancement of routine forensic practices with the evolving trend of abuse of these drugs.

### Stimulants, Oral Fluid, LC/MS/MS

## K47 An Assessment of Biological Matrices for the Detection of *In Utero* Cannabis Exposure

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the best biological matrices and biomarkers for detecting cannabis use during pregnancy and child exposure.

**Impact Statement:** This presentation will impact the forensic science community by presenting evidence of the best analytical tools to detect child cannabis exposure during pregnancy.

Cannabis consumption has been increasing worldwide among pregnant women. According to the 2017 National Survey on Drug Use and Health, 7% of pregnant women used cannabis in the past month, a 1.3% increase from the 2014 survey. As a result of the increase in legalization and decriminalization of marijuana at the state level across the United States, this upward trend is expected to continue rising. Due to the negative effects of prenatal cannabis exposure, it is necessary to develop an objective, sensitive, and specific method to determine cannabinoids use during pregnancy and child exposure.

This study compared four different biological samples—maternal hair, meconium, umbilical cord, and placenta—for the detection of *in utero* cannabis exposure. The biological samples were collected from 627 mother–newborn dyads. All hair and meconium samples were analyzed, and umbilical cord and placenta if hair and/or meconium were positive for cannabinoids. Maternal hair samples were divided into three segments corresponding to the three trimesters of pregnancy, when possible. All analysis were performed by liquid chromatography/tandem mass spectrometry-validated procedures.

Among the 627 cases, 32 cases (5.1%) tested positive for cannabinoids in maternal hair and/or meconium. Meconium and hair showed to complement each other, with an agreement between hair and meconium results of 96.7%, but only 34.3% if just positive results were considered. Umbilical cord and placenta results showed a better agreement with meconium (91.3% and 92.6%, respectively) than with hair (39.1% and 34.6%, respectively). Tetrahydrocannabinol (THC) hair concentrations ranged from 51.3 to 1,002.8pg/mg. The predominant metabolites in meconium were 11-nor-carboxy-THC (THCCOOH) and 8,11-dihydroxy-THC (diOHTHC), and in umbilical cord and placenta was THCCOOH-glucuronide. Cannabidiol (CBD) and Cannabinol (CBN) were detected in meconium but not in any umbilical cord or placenta. Cannabinoids' concentrations in meconium were higher (up to 887.4ng/g) than in placenta and umbilical cord (up to 28ng/g).

For the first time, prenatal marijuana exposure was analyzed and compared in paired hair, meconium, umbilical cord, and placental samples. Hair and meconium positivity rate was similar, but a more sensitive and specific analytical method for the hair may resolve discrepancies between the matrices. Umbilical cord and placenta may be considered suitable alternative matrices to meconium through the determination of THCCOOH-glucuronide as a biomarker of cannabis exposure.

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### Cannabis, Hair, Meconium



## K48 An Evaluation of Three Extraction Techniques for the Analysis of 11 Drugs and Metabolites in Authentic Hair Reference Material

*Brianna Spear, MS\*, Florida International University, Miami, FL; Anthony DeCaprio, PhD, Florida International University, Miami, FL*

**Learning Overview:** After attending this presentation, attendees will understand which of three commonly used techniques is optimal for extraction of each of a variety of abused drugs and metabolites from hair.

**Impact Statement:** This presentation will impact the forensic science community by presenting the most effective extraction techniques for analysis of oxycodone, diazepam, nordiazepam, methamphetamine, cocaine, cocaethylene, norcocaine, p-hydroxycocaine, morphine, 6-acetylmorphine, and fentanyl in hair and by providing data toward creating standardized procedures for hair testing.

**Hypothesis:** The hypothesis of this work is that different drugs will be extracted most effectively from hair using different extraction techniques, based on the physicochemical properties of the drugs of interest.

There are many differing opinions regarding the optimal methods for forensic hair analysis, especially concerning pretreatment and extraction parameters. Extraction methods include enzymatic, acid/base, and solvent techniques, each with their own advantages and disadvantages. The Society of Hair Testing (SoHT) suggests that any extraction technique can be utilized with the use of adequate control samples. This lack of consensus regarding best practice extraction methods contributes to possible bias in hair testing across multiple laboratories. Previous research in this laboratory has focused on pretreatment parameters, such as hair particle size, extraction solvent to sample size ratio, and extraction time. However, published work has not assessed the best extraction techniques for different drugs and metabolites of interest.

Authentic Hair Reference Material (HRM) containing the drugs of interest was obtained from a commercial source. Samples of 20mg each were weighed into steel milling jars. The hair samples were pulverized into a powder using a Retsch MM200 ball mill with chrome-steel milling beads at 3,800rpm for 30s. The samples were extracted using one of three techniques: a 2-h incubation in 12.5  $\mu\text{L}/\text{mg}$  hair of methanol:acetonitrile:2mM ammonium formate (25:25:50, v/v/v) at 37°C (solvent swelling technique), a 2-h incubation in 12.5  $\mu\text{L}/\text{mg}$  hair of 1M NaOH at 37°C (base technique), or a 2-h incubation in 12.5  $\mu\text{L}/\text{mg}$  hair of aqueous 12mg/mL dithiothreitol and 2mg/mL proteinase K at 37°C (enzymatic degradation technique). The samples were then transferred into Eppendorf tubes and centrifuged for 30min. Post-centrifugation, the eluent was subjected to solid phase extraction using an Agilent® Bond Elut LRC mixed mode C8 and Strong Cation-Exchange (SCX) cartridge. An Agilent® 1290/6460 Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) was used for analysis.

Recovery of each drug by each extraction technique was evaluated to determine which methods were most effective. Those with the highest extraction recovery included solvent swelling (for diazepam, nordiazepam, cocaine, p-hydroxycocaine, oxycodone, and 6-acetylmorphine), base (for fentanyl), and enzymatic degradation (for methamphetamine, cocaethylene, norcocaine, and morphine). In conclusion, the most effective extraction technique for removing drugs/metabolites from authentic HRM varies based on the physicochemical properties of the drugs of interest.

### Forensic Hair Analysis, Extraction Techniques, Authentic Hair Reference Material

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## K49 Fantastic Cases and Where to Find Them

*Katherine Dozier, PhD\**, Armed Forces Medical Examiner System, Dover, DE; *Bryan Platt, MD*, Armed Forces Medical Examiner System, Dover, DE; *Eric Berg, MD*, Armed Forces Medical Examiner System, Dover, DE and *Dept of Pathology Blanchfield Army Community Hospital, Ft Campbell, KY*

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**Learning Overview:** After attending this presentation, attendees will be familiar with the problem-solving approach required for difficult, non-standard forensic toxicology cases. Attendees will also understand the importance of fully utilizing all testing options available to report the forensically correct, scientifically accurate results.

**Impact Statement:** This presentation impacts the forensic science community by demonstrating the thought process required for difficult, non-standard forensic toxicology cases, and provides insights that can be applied to all forensic casework.

The Division of Forensic Toxicology at the Armed Forces Medical Examiner System (AFMES) is the only military forensic toxicology laboratory that can test postmortem, investigative, drug-facilitated sexual assault, Driving Under the Influence/Driving While Intoxicated (DUI/DWI), and other case types. Due to the unique mission of AFMES, submissions are received from military personnel stationed around the globe. Primary matrices are blood and urine, but there is the capability to test and report from tissue, vitreous, and more.

**Case 1:** A drunk-on-duty investigation provides multiple blood specimens, along with a urine specimen. However, upon testing, the one sodium fluoride blood tube confirms for ethanol, but there is no ethanol present in the urine. After a different blood tube is tested and confirmed positive for ethanol, the laboratory explores the possibility of the oxidative agents in the urine. The urine specimen is sent for validity testing and found to be valid, with no oxidative agents present.

**Case 2:** The laboratory's synthetic cannabinoid confirmation method is validated for blood and urine. However, an exsanguination case that screened presumptively positive for synthetic cannabinoids leaves the laboratory with no blood or urine specimens for confirmation. An examination of the available specimens yields a bloody tissue sample that holds the answers the laboratory seeks.

**Case 3:** A decedent is found unresponsive and later dies in the hospital after prolonged treatment. None of the specimens give any clues to cause or manner of death. However, a shirt covered in gastric contents was also found at the scene, and later provides the information that the laboratory needs in order to help the forensic pathologist determine cause and manner.

This presentation will examine each of these cases and the problem-solving necessary to effectively report the forensic toxicology results. As new cases are submitted to AFMES from around the world, the laboratory is poised to continue problem-solving.

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### Postmortem, Ethanol, Synthetic Cannabinoid

## K50 Quantifying the Relationship Between 1-Propanol and Postmortem-Formed Ethanol

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**Learning Overview:** After attending this session, attendees will better understand the risk of postmortem ethanol formation, how to identify it, and how to interpret postmortem ethanol concentrations.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with means to identify and quantify postmortem formation of ethanol using two independent markers of postmortem ethanol formation, 1-propanol and ethylsulphate.

Analysis of ethanol and other alcohols is routinely performed as part of the postmortem investigation. However, the detection of ethanol does not necessarily reflect ingestion since it can be formed during the decomposition process when fungi and bacteria start to ferment the available substrates to produce energy. During this process, in addition to ethanol, acetaldehyde, butanol, and propanol can also be produced. A common approach to diagnose postmortem ethanol is to analyze two matrices, preferably femoral blood and vitreous. The ethanol concentration in vitreous usually is slightly higher than the one in blood as a result of the matrix water content. Another approach is to analyze for propanol and butanol as markers for postmortem formation. A third means is to analyze the direct ethanol metabolite, Ethyl Sulphate (EtS), that is only formed *in vivo*. A negative result for EtS excludes ingestion of ethanol.

The objectives of this study were to develop and validate a method for the quantitation of methanol, ethanol, acetone, isopropanol, propanol, and butanol and to investigate the relationship between propanol concentration and ethanol concentration in EtS negative cases.

Alcohols were analyzed by head space Gas Chromatography/Flame Ionization Detector (GC/FID). Samples were prepared by dispensing 0.1mL blood, 0.5mL water containing internal standard *t*-butanol, and 0.5mL saturated potassium carbonate solution in head space vials. Calibration ranged from 0.01mg/mL to 2.5mg/mL for methanol, ethanol, acetone, and isopropanol and from 0.001mg/mL to 0.25mg/mL for propanol and butanol.

EtS was analyzed qualitatively by Supercritical Fluid Chromatography (SFC) and mass spectrometry. After the addition of deuterated internal standard, 0.5g of blood was prepared by protein precipitation with 1mL ice-cold methanol. Threshold for EtS was 0.025µg/g. Mobile phase A was carbon dioxide and phase B was 0.1 % ammonia in 90/10 methanol/water with a flow rate of 2.5mL/min. Sixty-four cases with suspected postmortem ethanol formation were included. Of the cases, 41 were negative for EtS but contained ethanol up to 1.35mg/mL, with 11 cases above 0.50mg/mL. All cases but two cases were positive for propanol (0.002–0.105mg/mL). The coefficient of determination between propanol and ethanol in all cases was 0.12 but 0.39 when only EtS negative cases were included.

This study concludes that propanol explains around 40% of the variation of ethanol in cases where all ethanol was formed postmortem. It also concludes that at least 1.35mg/mL of ethanol can be produced postmortem.

### Postmortem Ethanol Formation, 1-Propanol, Ethylsulphate

## K51 Branching Out: A Report of a Fatal Intoxication Case Involving 2-Methyl-2-Butanol

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the abuse of 2-methyl-2-butanol, a substance abused as an alternative for ethanol or benzodiazepines. Additionally, this presentation will describe an effective analytical method developed and validated in the laboratory to identify and quantify 2-methyl-2-butanol.

**Impact Statement:** The forensic science community will benefit from the reporting of an uncommon finding and be alerted about its potential for abuse.

Tert-Amyl Alcohol (TAA) or 2-methyl-2-butanol is a tertiary alcohol commercially used as an intermediate in organic synthesis and as a solvent for a wide variety of industrial products such as paint removers, among others. TAA is also a metabolic product of the O-demethylation of Tert-Amyl-Methyl Ether (TAME), both used as a gasoline oxygenate additives, and with psychoactive effects.<sup>1,2</sup>

2-methyl-2-butanol has originally been used as an anesthetic and recently reported in forensic case investigations as a recreational substance due to its benzodiazepine and ethanol-like effects. Additionally, this alcohol is easy to obtain, affordable, and usually not detectable via routine screening analysis. A case report of a 28-year-old male that attempted suicide using TAA quantified 83µg/mL of TAA in the blood, deep coma, respiratory failure, and tachycardia as the main observed symptoms.<sup>3</sup>

Pharmacological data is scarce in the scientific literature for TAA. However, *in vitro* experiments estimated a potency of TAA at the GABAA receptors around 10–20 times higher than that of ethanol.<sup>2</sup>

The aim of this study is to report the second case received in the laboratory where TAA was detected, and the analysis used in the laboratory to quantify this substance in the blood sample.

Quantitative determination of TAA in blood was approached as a custom assay development with supporting validation data. Blank patient samples (no standard added) were prepared diluted and neat in tandem with three fortified levels of standard addition at 0.5, 1.0, and 5.0µg/mL. Fifty µL of 0.00625% v/v 1,4-Dioxane was added as the internal standard. Analysis of the sample was performed by headspace Gas Chromatography/Mass Spectrometry (GC/MS) method using Single Ion Monitoring (SIM) mode: quantifier ion 73 and qualifier ions 59,71.

In the presented case received in May 2021 from New Jersey, a 37-year-old male with a history of substance abuse (heroin and alcohol) was found deceased without an obvious cause of death. The toxicological findings based on the originally requested testing showed only the presence of 13mg/dL acetone in the blood; further analysis of beta-hydroxybutyric acid was none detected, indicating that alcohol/diabetic ketoacidosis was not likely contributory to the death. Additional police investigations revealed the presence of liquid bottles labeled as “2-methyl-2-butanol” 97.5% purity, which was subsequently confirmed in the blood sample of the decedent. No anatomical findings were reported from the autopsy. The cause of death was attributed to fatal intoxication of TAA with a concentration measured in femoral blood of 1,000µg/mL.

**Conclusions:** This study describes an authentic TAA-related intoxication and the development and validation of an analytical method to quantify this substance of abuse.

### Tert-Amyl Alcohol, 2-Methyl-2-Butanol, Intoxication

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## K52 Fatal Intoxication Involving 2-Methyl AP-237: A Case Report

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**Learning Overview:** The goal of this presentation is to disseminate information on the Novel Synthetic Opioid (NSO) 2-methyl AP-237.

**Impact Statement:** This presentation will impact the forensic science community by increasing the awareness of 2-methyl AP-237 in fatal drug overdoses.

**Introduction:** In the United States, the opioid epidemic continues with NSO leading as the driving force. NSO are categorized as either fentanyl analogs or non-fentanyl analogs. Previously, fentanyl analogs were the most prevalent NSO, but subsequent to the scheduling of fentanyl-related substances in 2018, there has been a notable increase in non-fentanyl analog NSO. Recently, cases have emerged involving 2-methyl AP-237. This NSO is an analog of bucinnazine, also known as AP-237, which is an opioid that has similar potency to morphine. Presented here is a fatal intoxication case involving 2-methyl AP-237.

**Case History:** A 54-year-old White male was found deceased due to a possible drug overdose. The decedent, who was a transient, was found lying supine under a tree. Paramedics arrived and pronounced the decedent. The decedent was cool to the touch and had signs of rigor mortis in his extremities. There was a plastic container labeled “2MAP” and a cut straw found in the decedent’s backpack. There was a white powder found in the plastic container and straw that later tested positive for fentanyl by field testing. The decedent had a history of depression, shoulder pain, and early signs of dementia. Medical history revealed the decedent was treated for a drug overdose in October 2018. The case was pended for toxicological analysis.

**Results:** The case was submitted by the medical examiner for drug analysis. The Gas Chromatographic drug screen utilizing Nitrogen Phosphorous Detection (GC/NPD) revealed an unknown substance in the blood and urine. Further analysis by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) confirmed the presence of 2-methyl AP-237. Quantitation by GC/NPD was performed. The concentrations of 2-methyl AP-237 in the blood and urine were 480ng/mL and 4,200ng/mL, respectively. Additionally, alprazolam was detected in the blood at 55ng/mL.

**Conclusion:** The identification and quantitation of the NSO 2-methyl AP-237 was determined. There is minimal literature available regarding fatal intoxications involving 2-methyl AP-237. This case is illustrative of a lethal concentration of 2-methyl AP-237, considering the only other drug detected was alprazolam.

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### 2-Methyl AP-237, Synthetic Opioids, Postmortem

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## K53 Drug Taxonomy in Forensic Science Data for Integration Into Medicolegal Death Investigation Systems

*Alex Krotulski, PhD\*, CFSRE, Willow Grove, PA; Donna Iula, PhD, Cayman Chemical, Ann Arbor, MI; Agnes Winokur, MS, DEA, Miami, FL; Jeri Roper-Miller, PhD, RTI International, Research Triangle Park, NC; Bruce Goldberger, PhD, University of Florida, Gainesville, FL*

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**Learning Overview:** After attending this presentation, attendees will be able to explain the challenges associated with drug taxonomy and its incorporation into medicolegal death investigation systems.

**Impact Statement:** This presentation will impact the forensic science community by laying the framework for how drugs are named and communicated in the United States and, ultimately, how the information effects forensic sciences data exchange and stakeholder processes.

**Introduction:** The National Institute of Justice (NIJ), in partnership with its Forensic Technology Center of Excellence (FTCoE, 2016-MU-DX-K110) at RTI International, and the Center for Disease Control and Prevention (CDC), convened a virtual Medicolegal Death Investigation Data Exchange Working Group (MDI-Data-WG) over a 12-month period beginning in September 2020. The MDI-Data-WG was formed to advance forensic science and ensure communication between Medical Examiners and Coroners (ME/C), death investigators, forensic scientists, and other stakeholders.

**Objective:** A subcommittee of the MDI-Data-WG focused on methods of capturing and disseminating information on the types of drugs involved in deaths, including drug taxonomy and other categorizations, and classification needs concerning drug naming, drug terms, drug mappings, and drug classification. The goal is to disseminate information to all relevant stakeholders to facilitate information exchange of data related to drug overdose mortality.

**Methods:** The MDI-Data-WG consisted of more than 40 stakeholders (e.g., data producers like ME/C, data consumers like federal agencies) who participated in further formed Subcommittees (SC) that focused on a specific purposes and goals (e.g., drug taxonomy). The MDI-Data-WG met across five two-day meetings and intermittent SC monthly meetings. Approaches to collecting information included user case scenarios and feedback stakeholder types; presentations from invited guests with Q&A sessions; group experiential sharing; and forums with data programmers and technology developers. The work of the MDI-Data-WG resulted in a final report published by NIJ's FTCoE and CDC to include guidance to move toward consistency, data standards, and best practice for improving the process. The efforts of this working group will result in various user case profiles, presentations, implementation forums, further research, and process mapping and evaluations of the various processes.

**Results:** This presentation will focus on the work of the drug taxonomy SC directed on nomenclature of toxicological substances that may be identified by a laboratory to streamline the reporting of those substances by forensic pathologists, chemists, toxicologists, and others. The MDI-Data-WG produced guidance on naming conventions for newly emergence synthetic drugs and developed a flow chart to assist with the understanding of complex drug taxonomy. The working group reviewed and discussed online information management systems that would benefit forensic scientists as they relate to drug nomenclature and tracking, and outlined what information should be collected, consolidated, and archived. Through interactions among the SC and with others in the forensic science community, the working group provided recommendations for drug taxonomy and ideas for how to maintain an online database in real-time.

**Conclusion:** Drug taxonomy remains a vital component for communication and information sharing within the medicolegal death investigation systems. Forensic data are often queried and reported based on findings listed on death certificates, toxicology reports, and other data streams. To facilitate proper data exchange and communication, the forensic community needs standardized data and a common drug language, especially one that is adaptable and updated in real-time as emerging drug threats appears. The work of the MDI-Data-WG will be documented in a final report to be available on the FTCoE website ([www.forensicCOE.org](http://www.forensicCOE.org)).

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### Medicolegal Death Investigation, Taxonomy, Nomenclature

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## K54 Postmortem Polysubstance Use Trends in Tramadol-Positive Cases From 2015–2020 in Pennsylvania

*Stephanie Marco, PhD\*, NMS Labs, Horsham, PA*

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**Learning Overview:** After attending this presentation, attendees will understand how trends in tramadol-positive postmortem toxicology have changed in Pennsylvania since 2015.

**Impact Statement:** This presentation will impact the forensic science community by providing new information on polysubstance use trends in tramadol-positive postmortem toxicology cases.

**Introduction:** Tramadol is a central nervous system analgesic commonly prescribed for the treatment of moderate to severe pain. Recently, reports of tramadol overdoses have increased, with the primary cause of intoxication being suicide followed by abuse and unintentional intoxication. Additionally, tramadol has been detected in seized street drug mixtures containing heroin, fentanyl, methamphetamine, synthetic cannabinoids, and cocaine. Polysubstance use involving tramadol and other common drugs of abuse can increase the risk of adverse effects and fatal complications. This study identifies trends in drug positivity and polysubstance use in tramadol-positive postmortem blood.

**Methods:** Postmortem blood specimens submitted for analysis by Pennsylvania-based death investigators between January 2015 and December 2020 were studied. Drugs of abuse including barbiturates, benzodiazepines, cannabinoids, ethanol, hallucinogens, hypnotics/sedatives, inhalants, opioids, and stimulants were identified in cases positive for tramadol and/or its metabolite O-desmethyltramadol. Specimens were screened by Enzyme-Linked Immuno-Sorbent Assay (ELISA), Liquid Chromatography/Time of Flight/Mass Spectrometry (LC/TOF/MS), and/or headspace Gas Chromatography (GC). Confirmation testing was performed using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS) and/or Gas Chromatography/Mass Spectrometry (GC/MS).

**Results:** Between 2015 and 2020, 771 cases met the study criteria. The number of tramadol-positive cases ranged from 89 to 157 per year. From 2015 to 2020, stimulants (+23%) and benzodiazepines (-10%) had the highest total percent change in positivity rates. The most pronounced yearly increase for analyte positivity was observed for opioids (+38%) between 2019 and 2020, whereas the highest decrease in analyte positivity occurred for opioids (-33%) between 2018 and 2019. Except for 2019, opioids were the most frequently identified analytes in tramadol-positive postmortem cases, with 2015 having the highest percent positivity at 77%. Fentanyl was consistently the most positively identified opioid from 2016 to 2020, whereas oxycodone had the highest percent positivity in the opioid class in 2015. In 2019, benzodiazepines were more frequently detected than opioids (42% vs. 36%), with alprazolam having the highest positivity rate in the benzodiazepine class. Barbiturates, hypnotics/sedatives, and inhalants were the least frequently identified drug classes in tramadol-positive cases during the study period (all  $\leq 1\%$ ).

**Conclusions:** Over the past five years, there has been a modest increase in polysubstance use associated with tramadol-positive postmortem cases in Pennsylvania. The popularity of opioids and benzodiazepines in these cases has been the most consistent throughout this period, although the positivity rates varied, especially for opioids. The rise in stimulant positivity in tramadol-positive cases during the study period could be due to increased drug experimentation among tramadol users or the increased presence of tramadol as an adulterant in street drugs. Further studies may help elucidate the cause(s) of these findings.

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### Tramadol, Trends, Postmortem

## K55 A 3D Origami Paper-Based Microfluidics Device for Creatine Analysis in Urine: A Disposable Tool for Identifying Urine Sample Adulteration by Dilution

Giacomo Musile, PhD\*, University of Verona, Verona, Italy; Yvane Agard, MS, University of Verona, Verona, Italy; Sara Pesavento, University of Verona, Verona, Italy; Matilde Murari, MS, University of Verona, Verona, Italy; Elio De Palo, PhD, University of Verona, Verona, Italy; Franco Tagliaro, PhD, University of Verona, Verona, Italy and Sechenov University, Moscow, Russian Federation

**Learning Overview:** Attendees will receive detailed information on the use of a novel technique to control the integrity of urine samples using paper-based microfluidics technology. In the present case, this technique was applied to the analysis of creatinine, the most common analyte to control urine adulteration by dilution.

**Impact Statement:** Because of the low cost and portability of the developed devices, this method has a potential application outside the use of specialized laboratories and may be used to control urine integrity immediately at the site of collection in doctor's offices, in small laboratories, and/or in occupational medicine centers.

The relevance of the problem of sample tampering is well-known in forensic toxicology, and sample dilution is the most used method to cheat toxicological controls. The prevalence of this phenomenon, with a long history in analytical toxicology, is reported also in recent papers.<sup>1-3</sup> Among the criteria to assess urine integrity by excluding dilution, the quantification of creatinine probably represents the most popular method.

Although the majority of analytical methods offer adequate sensitivity and specificity, at present, creatinine analysis requires laboratory instrumentation, which hinders the possibility of direct test of urine at the collection site. This hinders the immediate interaction with the urine donor, which could be important to prevent claims of post-collection counterfeiting.

Since the first introduction by Whitesides et al., the use of Paper-based microfluidic Devices ( $\mu$ PADs) for the development of chemical sensors has been extensively reported.<sup>4,5</sup> Among several approaches for producing  $\mu$ PADs, the use of commercial wax printers proved to be inexpensive and straightforward in the fabrication of the device. The procedure is based on two steps: (1) patterning chromatography paper into hydrophilic channels by fabricating hydrophobic barriers, and (2) addition of the reagent to the hydrophilic portion of the paper support. The sample is driven through the reagent zone as a result of the wicking capacity of the paper without external assistance. This approach does not require highly qualified personnel nor expensive instrumentation. Also, it can be performed on-site, enabling a prompt analytical response also in less-equipped environments. The advantages of  $\mu$ PADs have provided forensic science with reliable tools to face different forensic issues.

On grounds of the above considerations, the goal of the present work was to develop a low-cost device able to provide a rapid and sensitive colorimetric detection of creatinine in urine samples. This presentation will provide details of the developed procedure, which was conceived as a first-line screening potentially to be confirmed with laboratory instrumentation.

The proposed microfluidic devices were designed as a 3D origami pattern. The test was based on three specific reactions for the detection of creatinine using picric acid, 3,5-dinitrobenzoic acid, and Nessler's reagent. The urine sample was transferred without any treatment directly onto the hydrophilic portion of the paper and colorimetric reactions are developed in few minutes. The color change was measured in terms of "RGB distance" by using a simple and free software for smart phone cameras. The device was also validated for quantitative determinations in terms of accuracy and precision. The optimized method was tested on real urine samples ( $n=53$ ) using as reference a clinical chemistry method performed on immunoassay instrument.

In conclusion, the perspective usefulness of paper-based microfluidics as a low-cost and easy to use technique in forensic toxicology will be presented with a specific focus on its possibilities of on-site analysis to prevent urine adulteration.

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### Microfluidic Paper-Based Devices ( $\mu$ PADs), Urine Adulteration, Forensic Toxicology



## K56 The Evaluation and Laboratory Testing of Synthetic Urines and Urine Adulterants Available in the United States

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**Learning Overview:** Attendees of this presentation are expected to learn about the current state of synthetic urines and urine adulterants and how they impact workplace drug testing.

**Impact Statement:** This presentation will impact the forensic community by informing laboratory directors and other decision makers about the current state of synthetic urines and urine adulterants, allowing them to better interpret data from urinary testing and to evaluate their methodology.

Urine drug testing has been the standard in both military, federally mandated, and non-regulated workplace drug testing for more than 30 years. However, as the impacts of a positive drug test often are severe for the donor, there has always been an incentive to “beat the test.” In the past decade, a number of commercial manufacturers have specialized in products designed to subvert urine drug testing, including synthetic urines that replace the sample, adulterants that interfere with testing methods, and various contraptions to hide the former from the collector. To identify the use of these products, the collector measures the temperature of urine specimens at the time of collection and the laboratories employ various Specimen Validity Tests (SVT), as discussed below.

An online market survey conducted in December 2020 identified 32 synthetic urine products and 3 urine adulteration products. Based on the survey, 16 synthetic urine products and all 3 urine adulterants were purchased and analyzed in-house and by five Health and Human Services (HHS) -certified reference laboratories. Adulterants were added to negative urine and urine containing amphetamine, methamphetamine, benzoylecgonine, codeine, morphine, PCP, THC-COOH, and synthetic opioids at concentrations approximately two times the federally mandated cutoff levels. In-house analyses included visual inspection and SVT using dip sticks. Laboratory testing was conducted on ten synthetic urine products, all three adulterants and control urine samples. Included were sample validity testing (pH, oxidants, creatinine, and specific gravity), immunoassay screening, and confirmation of spiked drug concentrations.

Visual inspection revealed that most products were professionally packaged, indicating the size and maturity of the industry. Also, both the synthetic urines and the adulterated urine samples would be difficult to distinguish from authentic urine specimens based on their appearance. Except for one sample with low specific gravity, all synthetic urine samples passed mandated laboratory SVT tests for creatinine, specific gravity, oxidants, and pH. However, specific gravity is normally not tested on specimens with normal creatinine levels.

The first adulterant was clearly identified by general oxidant tests, in line with manufacturer warnings that it was detectable. It also interfered with the internal standard of one confirmation assay. The second adulterant was undetected by SVT testing, but did not affect any drug test results. The last adulterant produced two negative screening results in one laboratory and lowered the quantitative value of some drug analytes. However, the adulterated sample was identified as invalid by immunoassay suppression, loss of internal standards in confirmatory testing, and the iodate SVT test.

In summary, most synthetic urines would have been difficult to identify at the laboratory using current testing protocols, but several laboratories are working on novel methods to identify urinary biomarkers. It is harder to subvert a urine drug test with an adulterant as the only adulterant that was not identified by SVT testing did not affect the drug test results.

### Synthetic Urine, Adulterant, Workplace Drug Testing

## K57 Respiratory Depressant Effects and Pharmacokinetics of Oral Mitragynine (Kratom) and Oxycodone in Rats

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**Learning Overview:** Attendees will be able to better compare the respiratory depressant effects and relative toxicity of oral mitragynine and oxycodone.

**Impact Statement:** Lack of respiratory depression following high-dose mitragynine in rats questions the appropriateness of designating kratom as cause of death in postmortem cases.

Mitragynine, the primary active alkaloid in kratom and partial  $\mu$ -opioid receptor agonist, has substantially different pharmacology from the full  $\mu$ -opioid receptor agonists morphine and oxycodone. Mitragynine does not recruit  $\beta$ -arrestin for G-protein signaling, has activity at adrenergic receptors, and a lower risk of respiratory depression, acute poisoning, death, and abuse-related effects. The kratom alkaloid, 7-Hydroxy-Mitragynine (7-OH-MG), constitutes <1.5% of the total alkaloid content, is more potent at the opioid receptor than mitragynine, and a mitragynine metabolite. Mitragynine is not scheduled in the Controlled Substance Act; however, the Food and Drug Administration (FDA) recommended scheduling of mitragynine in 2016, in part out of safety concerns, though the request was rescinded by the Assistant Secretary of Health in 2018. Mitragynine is taken by millions of people in Southeast Asia and the United States as a mild stimulant and analgesic and to treat opioid dependence and withdrawal.

In 2020, the FDA recently recommended a rat model for evaluating drug respiratory depressant effects, utilizing oxycodone as a positive control. This study employed this model to compare respiratory depression following 20, 40, 80, 240, and 400 mg/kg oral mitragynine and 6.75, 60, and 150mg/kg oral oxycodone in rats. Oxycodone, mitragynine, and 7-OH-mitragynine were quantified by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) pre-dose, 1, 2, 4, 6, 8, and 12h after administration. Blood gases were determined at the same time points with the VetScan i-STAT Alinity V Handheld Analyzer. Clinical observations of opioid associated effects and toxicity were performed throughout.

A significant decrease in oxygen saturation compared to baseline sO<sub>2</sub> following the 60 and 150mg/kg oxycodone doses was observed, with no significant differences in sO<sub>2</sub> following 20, 40, 80, 240, and 400mg/kg mitragynine over 12h. Partial CO<sub>2</sub> pressure change from baseline was significantly increased after 60 and 150mg/kg oxycodone hydrochloride, but not following any mitragynine dose. Two animals receiving oxycodone, one at 60 and one at 150mg/kg, died after dosing, and 66.7% and 100% of animals receiving these doses had abnormal clinical observations. One animal following 240mg/kg and multiple animals following 400mg/kg mitragynine had abnormal clinical observations. Median blood oxycodone C<sub>max</sub> were 33.5, 39.9, and 131ng/mL following 6.75, 60, and 150mg/kg oxycodone, respectively, with T<sub>max</sub> at 1h. Median mitragynine C<sub>max</sub> were 723, 1,665, 2,140, 6,278, and 6,341ng/mL after 20, 40, 80, 240, and 400mg/kg mitragynine, respectively, with T<sub>max</sub> generally at 1h. 7-OH-mitragynine C<sub>max</sub> were 11.0, 17.4, 18.9, 32.0, and 62.5ng/mL after 20, 40, 80, 240, and 400mg/kg mitragynine, respectively, with T<sub>max</sub> at 1h. 7-OH-mitragynine concentrations were approximately 100-fold lower than mitragynine concentrations in the same sample.

Mitragynine doses as high as 400mg/kg did not produce any clinically relevant or significantly measurable effects on respiration based on blood gas measurement, whereas 60 and 150mg/kg oxycodone resulted in moderate to severe respiratory depression in rats. Similarly, there were no abnormal clinical observations after 20, 40, and 80mg/kg, one after 240mg/kg and multiple after 400mg/kg oral mitragynine, while 60 and 150mg/kg oxycodone produced many abnormal observations and two deaths.

**Conclusion:** Oral mitragynine had little respiratory depression in rats following up to 400mg/kg doses, much higher doses than generally taken by humans, suggesting caution in attributing cause of death to mitragynine respiratory depression in postmortem deaths.

### Mitragynine, Respiratory Depression, Pharmacokinetics

## **K58 The Application of All Ions Data Acquisition and SureMass Quantitative Data Analysis for Comprehensive Drug Screening Detection in Whole Blood**

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**Learning Overview:** After attending this presentation, attendees will understand the advantages of utilizing an All Ions Liquid Chromatography/quadrupole Time-Of-Flight (LC/qTOF) data acquisition approach for comprehensive toxicology drug screening analysis.

**Impact Statement:** This presentation will impact the forensic science community by presenting toxicological data analysis challenges.

Forensic toxicology serves the community through civil, criminal, and medicolegal investigations. The analytical burden associated with the detection of drugs of abuse, prescription medications, over-the-counter drugs, and new psychoactive substances in complex biological matrices (i.e., whole blood) is significant. The scope and sensitivity of toxicological screening is a challenge for many laboratories, and sample preparation is key in the performance of targeted or non-targeted assays.

Common sample preparation methods include protein precipitation, lipid removal, dilution, filtration and centrifugation, Liquid-Liquid Extraction (LLE), Supported Liquid Extraction (SLE), and Solid Phase Extraction (SPE) -based technologies, including dispersive SPE and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS). However, no single technique can produce high extraction efficiencies for hundreds of drugs with a wide range of physicochemical properties and removing all matrix interferences. Likewise, although chromatographic separation of all components is desired, this may not be achievable. Therefore, a practical compromise is often struck between assay performance and overall efficiency. This study reports how data analysis tools were used to mitigate some of these issues using All Ions data acquisition.

High-resolution mass spectrometry was used to identify >200 compounds in whole blood using SLE and LC/qTOF using All Ions acquisition. The comprehensive screening assay targeted drugs of interest in impaired driving and medicolegal death investigation at the recommended cutoffs.<sup>1,2</sup> The method was successfully validated in accordance with published guidelines.<sup>3</sup>

Later, a series of adjudicated case samples that had been stored for an extended period (i.e., years) were re-evaluated using the methodology herein described. These specimens displayed increased matrix interferences and background noise for selected compounds, including 11-nor- $\Delta^9$ -Carboxy-Tetrahydrocannabinol (THCA), a Tier I drug of importance. As a result, false negatives were obtained for samples that previously confirmed positive. New data analysis techniques were explored using Agilent® MassHunter Quantitative software with SureMass capability. The combination of All Ions acquisition and optimized data analysis was able to overcome the assay limitations due to the degraded matrix. Data collection at various collision energies and the elimination of abundance thresholds and projected ion transitions mitigated the false negative results.

This research shows the utility of the All Ions acquisition technique for analyzing complex biological matrices (i.e., whole blood) and for alleviating matrix effects from co-eluting interferences with innovative data analysis techniques.

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2. Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing for Medicolegal Death Investigations. ASB Draft Standard 119 (2019).
3. Standards Practices for Method Validation in Forensic Toxicology, ANSI/ASB Standard 036 (2019).

### **All Ions, LC/qTOF, Blood**

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## **K59 Field Impairment Testing: Establishing the Statistical Variability and Uncertainty of Measurement in the General Population**

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**Learning Overview:** Attendees can expect to learn about the differences in the United States and United Kingdom Field Impairment Testing systems, and the performance ability of the general public when considering individual factors such as sex, age, weight, and height. The attendees will further learn that even in complete sobriety, increased age and Body Mass Index (BMI) resulted in increased failed impairment tests. Finally, attendees will also learn that even though these tests have been previously validated, most participants negatively commented on the difficulty of the administered tests.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the problems associated with Field Impairment Tests, including the issues with validation linked with degree of impairment, the effects of independent characteristics, and difficulty associated with the tests success rate.

Field Impairment Testing within the policing system as a method for determination of the intoxication of an individual was examined in the United Kingdom and compared to the original United States Standardized Field Sobriety Tests.

In the United Kingdom, although police officers are trained to perform such tests on members of the public and to identify indications of impairment throughout each individual test, there is no standardized indication or clue number, unlike the United States' system. Thus, officers must subjectively decide on the impairment of the individual based on performance, leaving room for potential personal bias within the decision process. Despite police officers always seeking to obtain details of medical conditions as a reason for inability to perform, there is no current consideration on whether other factors such as sex, age, weight, or height will alter the outcome of these tests.

This raises questions regarding the ability of the individual being assessed, specifically if they would be classified as impaired when sober. This study carried out research to determine whether individual factors could influence the test outcome. Fifty-four volunteer participants completed all five Field Impairment Tests, and the results were collated dependent on sex, age, weight, and height.

The results suggest that impairment indication scores tend to increase with increasing BMI. The research also found significant variation in test results of participants aged 30+ in comparison to younger age groups. Additionally, correlation of elderly adults with increased likelihood of medical conditions was linked to failed impairment tests. Medical conditions resulted in increased likelihood of failed impairment tests over all age, sex, weight, and height groups. Difficulty of the tests were also highlighted, thus suggesting the tests are not fit for purpose and should be revised for use under Section 6 of the Road Traffic Act 1988 in the United Kingdom, in addition to suggestion for revision of the original scoring system used in the United States.

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### **Driving While Impaired, Field Impairment Testing, Statistical Variability and Uncertainty of Measurement**

## K60 Carfentanil and Xylazine: Veterinary Drugs Detected in Drug Abuse-Related Forensic Casework

Andrea Carolina Noble, PhD\*, NMS Labs, Horsham, PA; Donna Papsun, MS, NMS Labs, Horsham, PA; Barry Logan PhD, NMS Labs, Horsham, PA

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the increase in the use of two potent tranquilizers approved to be used in veterinary medicine, but extensively misused as a drug of abuse and/or as a toxic adulterant.

**Impact Statement:** This presentation will impact the forensic science community by presenting trends in the use of two veterinary drugs, xylazine and carfentanil, detected in routine forensic casework.

Xylazine and carfentanil are drugs commercially available to use in the veterinary field to tranquilize large animals, mainly used as horse anesthetics. These two drugs belong to different chemical classes; xylazine is a non-opioid sedative drug from the phenothiazine analog group that acts at the alpha-2-adrenergic receptors, while carfentanil is a fentanyl analog and a  $\mu$ -opioid receptor agonist.<sup>1,2</sup> Forensic investigations have reported these drugs have been extensively detected in illicit drug formulations, especially since 2017.<sup>3,4</sup>

Blood samples from forensic casework investigations were considered for this study. Two different routine Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) methods were used to determine xylazine and carfentanil, with a limit of quantification of 5ng/mL and 0.05ng/mL, respectively. For this study, only those samples received in the laboratory from 2020 and Q1 and Q2 of 2021 were considered to analyze the recent trends in the illicit use of carfentanil and xylazine.

Xylazine was confirmed in a total of 2,776 blood samples during the considered period. An average of 119 samples/month were confirmed for xylazine during 2020; while in 2021, the average was 192 xylazine-positive samples/month during January–June 2021. Twenty-five percent of the positive samples were received from Pennsylvania and 10% from Michigan in 2020. However, in 2021, 21% were from Pennsylvania, 13% from Connecticut, followed by New Jersey and New York at 6–7%. Peripheral blood mean concentration, median, and range were as follows: 29.9ng/mL, 16ng/mL, and 5–610ng/mL ( $n=1800$ ). The most prevalent co-occurring drug in those cases positive for xylazine was fentanyl (96%), delta-9 THC (27%), heroin, and methamphetamine (both 23%).

A total of 370 blood samples were positive for carfentanil from January 2020–June 2021, 220 in 2020 versus 150 in 2021 (Q1 and Q2), with an average of positivity of 8 (2020) versus 14 (2021) samples/month. Mean carfentanil peripheral blood concentration was 0.57ng/mL, median 0.23ng/mL, and range 0.051–5.9ng/mL ( $n=153$ ). For carfentanil, however, fentanyl was present in 45% of positive cases, delta-9 THC in 24%, methamphetamine in 30%, cocaine in 26%, and heroin in 22%. An increasing amount of methamphetamine positivity was observed in carfentanil-involved cases, a trend that has increased over the past four years, which is likely due to the growing use of methamphetamine by drug users.<sup>5</sup>

The increase in the illicit use of carfentanil and especially of xylazine has been alarming in 2020. These data show that intoxication cases where xylazine or carfentanil are involved are still growing in 2021. However, both drugs seem to have different roles: xylazine as an adulterant of the illicit use of fentanyl (so-called “tranq dope” mix); while fentanyl was not detected in 55% of the analyzed carfentanil positive blood samples and, within these cases, cocaine, heroin, and methamphetamine were the most prevalent drugs of abuse.

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### Drugs of Abuse, Toxic Adulterants, Forensic Toxicology

**LW1 A Forensic Analysis of the 1960 Rome Olympics' 100-Meter Dash Final**

*John D. Bullock, MD\*, Ophthalmic History Research Institute, Winchester, MA*

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**WITHDRAWN**

## LW2 Who Am I? What is My Name?

*Colleen M. Fitzpatrick, PhD\*, Identifinders International LLC, Fountain Valley, CA*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how modern research methods can illuminate the historical context of the Holocaust.

**Impact Statement:** This presentation will impact the forensic science community by describing the role played by multidisciplinary analysis in identifying Holocaust survivors and reuniting them with their families.

Of the 1,600,000 Jewish children who lived in Europe before World War II, only 100,000 survived the Holocaust. Most child survivors were hidden children, shuttered away in attics, cellars, convents, or farms. Unfortunately, in the chaos that reigned after Europe was liberated, restoring children to their birth families was especially difficult—many parents perished in the camps, and children were often too young to know their own names. Today, there are still hundreds of child survivors who have been unable to recover their birth identities.

This presentation is about efforts to restore the identity of just one of those children—Pnina Gutman, a 74-year-old great grandmother now living in Israel. Pnina was smuggled from the Warsaw Ghetto in 1942 when she was an infant. She has lived her life with no knowledge of who she is.

Pnina's life was saved by Charlotte Rebhun, a Christian woman from Berlin whose Jewish husband had just been arrested in Warsaw and sent to Treblinka. What little we know of Pnina's rescue from the Ghetto comes from the eyewitness accounts Charlotte's children Adele and Wolfgang gave to the Yad Vashem when they were reunited with Pnina in 1997:

*It all started with a conversation between Mrs. Gertrude Spyra, wife of a Jew from Berlin, and our mother, Charlotte Rebhun born Schössow. The conversation involved the adoption of a small child of a Jewish family from the Warsaw ghetto—the safekeeping and the survival of the child until its retrieval after surviving threats to life and limb. The birth parents were organized resistance fighters in the Warsaw ghetto.*

*The handover of Baschke by the birth mother took place in our former apartment in Warsaw Krochmalna No. 33, and included a baby stroller with baby clothing. The intention was presented to my mother, that after taking care of their responsibilities in the Ghetto, the parents would return for the child after not too long a period of time.*

*After that, in the fall of 1942—the exact time, unfortunately, does not come to mind—there was no sign of the parents. Barbara's parents were Polish Jews.*

*In daily life, Barbara was our child and our sister, with the name Barbara Rebhun.*

The story continues from there with only one other small, yet highly significant, clue. According to the Rebhuns, upon her departure from their apartment that day in 1942, Pnina's mother counselled Charlotte, "If we don't come back for our daughter, send her to our relatives in America." That was only weeks before the Warsaw Ghetto uprising in April 1943.

Research into Pnina's identity has been both difficult and rewarding. The search has been global; Holocaust records, like Holocaust survivors, are scattered throughout the world. So many questions have been answered, yet there are so many more. Thanks to direct-to-consumer DNA testing, those relatives in America described by Pnina's still anonymous mother have been discovered. One of Cousin Helen's aunts was Pnina's grandmother. Yet which aunt, and who did she marry? This presentation discusses how even Helen's collection of family photos, some with names on the back, has brought the solution to the mystery of Pnina's identity so much closer, and yet her identity still remains frustratingly just beyond reach.

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### Holocaust, Survivor, DNA

## LW3 Who You Gonna Call? Forensic Scientists as Responsive and Responsible Practitioners

*Matteo Borrini, PhD\*, Liverpool John Moores University, Liverpool, England, United Kingdom; Marco Marocutti, Cicap, Padova, Italy*

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**Learning Overview:** After attending this presentation, attendees will be informed regarding how being responsive not only means reacting quickly and being ready to face the challenges proposed by the modern crime scene, it also means being responsible and reliable in way that the experts select and using the appropriate tools during forensic investigations.

**Impact Statement:** This presentation will impact the forensic science community by providing examples regarding the importance of understanding which equipment is the most appropriate for specific needs by knowing the potential of each tool, as well their limitations.

The word “modern” associated with “science” brings to mind the idea of limitless equipment with super-powerful equipment that can help scientists in their daily challenges. However, scientists have an obligation to be responsive and reliable, and these characteristics can only be achieved by a wise and responsible use of the available tools. The selection and application of the appropriate equipment can only be achieved if the users know the potential of the available methods and, more importantly, their limitations and weaknesses.

To better explain this concept, this presentation will discuss the paradox offered by alleged scientific disciplines that often try to equalize themselves to the forensic world: ghost hunting. Ghost hunters, often self-defined as paranormal detectives, try to increase the credibility of their alleged discipline, portraying themselves as investigators focused on the search for evidence of spirits and disembodied entities.

Even if there are cases where the malevolent influence of spirits has been (unsuccessfully) proposed to explain the death of some individual or the aberrant actions of heinous homicides, this presentation aims to analyze the daily (and nightly) routine of ghost hunters and their approach to scientific equipment. In addition to carrying shining badges and paramilitary uniforms, modern ghostbusters attempt to appear professional by using sophisticated equipment that people would expect to be employed by forensic investigators in very complex crime scenes. However, the reality is rather different.

One of the most iconic tools for ghost hunters is the electromagnetic field detector. The device is designed to detect, but not measure, electromagnetic fields. Consequently, the detector is a non-diagnostic analytical tool despite the misuse in ghost hunting expeditions. In these contexts, people are more interested in the emotions of “seeing something” rather than measuring and analyzing an event.

Thermographic cameras represent another example of misusing a tool. This kind of apparatus has been designed to make visible what usually is not detectable by the naked eye: temperature variations. In this case, ghostbusters are victims of a scientific oversimplification, assuming that if the camera can make noticeable what usually is not, it should achieve the same with what is supposedly impossible to be seen: spirits. In addition, thermographic cameras do not have high resolution, which helps create evocative stimuli in the users and, consequently, pareidolic effects.

The thermal imager offers food for thought also regarding the lack of habit with a non-common tool, stressing how it is essential to know all the equipment’s characteristics, potential, and weaknesses before bringing it into the field. A similar problem is encountered even with traditional photo cameras, where backscatters from unfocused motes of dust are classified as paranormal phenomena under the name of “orbs.” Forensic scientists are not misled by these artifacts. Nevertheless, as cameras are ubiquitous on the crime scene, it is still common to see some practitioners equipped with expensive and sophisticated digital reflex cameras using them in fully automatic mode—a clear example of a lack of knowledge of the limits and potential of the tool.

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### Pseudoscience, Ghost Hunting, Debunking



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## LW4 A Forensic Photo Mystery: The Railroad Picture

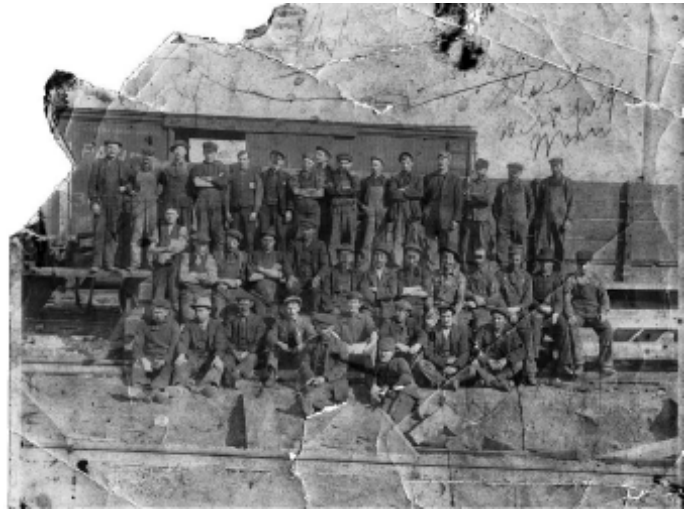
*Colleen M. Fitzpatrick, PhD\*, Identifinders International LLC, Fountain Valley, CA*

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**Learning Overview:** After attending this presentation, attendees will have learned how attention to detail is an important element in forensic photo analysis.

**Impact Statement:** This presentation will impact the forensic community by illustrating the value of a multidisciplinary approach for extracting information that can be used to answer the questions who, what, when, and why.

There was not much information supplied by the owner of this picture of a group of railroad workers from the early 20<sup>th</sup> century—only that it was taken in Canada, and that his Uncle Patrick Swords was one of the workers. Yet through careful observation, it has been possible to construct the narrative for Uncle Patrick after he arrived in Canada, and to derive much information about other workers in the picture along with the circumstances under which the photo was taken. The most obvious clue to the photo is the writing that appears across the top that can be deciphered as “Sherburn Street, Winnipeg, Man.” The writing on the boxcar in the left background is also informative. An investigation of the history of Winnipeg shows that by the late 1880s, the city had become a major hub for the Canadian Pacific Railroad. The word on the boxcar is “Pacific.” Another look at a map of Winnipeg shows that Sherburn Street dead ends close to the main depot of the Canadian Pacific Railroad. Winnipeg city directories from 1910–1914 quickly lead to a Michael Swords residing at 951 Sherburn Street, who worked as a laborer for the railroad. The 1915 city directory gives the listing at that address as “Kate, wid. Michael.” But is it possible to do more? Is it possible to identify any of the other men in the picture, if not by name, at least by occupation? Is it possible to deduce the time and approximate location the picture was taken and the reason? Through careful attention to detail, the answer is yes. Through observations of the clothing, the accessories, and the postures of the workers, it is possible to discover what positions they filled on the railroad crew. Other less-obvious details lead to an approximate time and date and the place the photo was taken, along with a good guess for the reason. Even the weather is a factor in developing a backstory for the photograph and to solve the mystery of its who, what, when, where, and why.



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### Forensic, Photograph, Analysis

### LW5 A Life for the Dead: In Memory of Bill Haglund (1943–2021)

*Constantine Eliopoulos, PhD\*, Liverpool John Moores University, Liverpool, England, United Kingdom*

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**Learning Overview:** After attending this presentation, attendees will have been informed about the life and contributions of Bill Haglund to the field of forensics and, in particular, the investigation of war crimes and human rights abuses.

**Impact Statement:** This presentation will impact the forensic science community by showcasing human rights investigations led by a leading figure in the application of forensic anthropology in the scientific excavation of mass graves.

Bill Haglund's life is a shining example of resilience and perseverance. Having an extremely difficult childhood and moving around a lot, he faced many challenges that all made him stronger and gave him a sense of empathy. When he was a teenager, his mother was stabbed to death in a Minnesota bar, a tragedy that led him down the forensic path later in his life. After graduating from high school, he worked at a funeral home and attended the California College of Mortuary Science. This was followed by a bachelor's degree in biology from U.C. Irvine. In the late 1970s, he started to work as a death investigator with the King County Medical Examiner's Office in Seattle, WA, and while at his post, he earned a PhD in physical anthropology from the University of Washington under the mentorship of Daris Swindler. It was around this time that he started working in the field of human rights as a consultant for investigations in the Balkans, Latin America, and Africa. He later took on roles such as senior forensic advisor to the United Nations' International Criminal Tribunals and served as Director of the international forensic program of the organization Physicians for Human Rights. His work took him to several countries, many of which were active war zones.

In the academic sphere, Bill Haglund published several articles on death investigation, but his main contribution is considered by many to be the volumes on forensic taphonomy that he edited with Marcella Sorg.

Bill was also an active member of several forensic associations and a Fellow of the American Academy of Forensic Sciences. He received the T. Dale Stewart award in 2013 recognizing his lifetime contribution to the field of forensic anthropology.

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**Forensic Anthropology, Human Rights, Mass Grave**

**Y1 DNA Integrity and Fungal Contamination as a Function of Time in Freshwater Drowning Deaths**

*Maeve Godshalk, BS\*, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** This presentation aims to better evaluate the current knowledge of the DNA and fungal relationship in a forensic setting, or lack thereof. Specifically, this research examines if human DNA is degraded due to fungi and the environment.

**Impact Statement:** This presentation aims to give forensic laboratories a better understanding of tissue contamination as it relates to fungus and improve decision-making in regard to processing such samples.

Drowning and drowning deaths are categorized as a public health issue. Fatal and potentially criminal drowning often results in the inability to process DNA evidence. Drowning deaths have a unique pathology, including wrinkling of the skin, skin slippage, putrefaction, and adipocere formation. The combination of these pathological changes with the freshwater environment allow for microbial and fungal degradation of the victim's tissue to occur at a hastened rate and make it difficult to process tissue samples. Forensic mycology, the study of fungi, is an emerging field and has been proven to be crucial in different cases.

In drowning deaths, forensic mycology can be used to evaluate the level of degradation to the victim and other evidence, as well as give insight into the health of the individual while they were alive. Fungal contamination can create issues during DNA processing and negatively affect the integrity of the sample. *Candida albicans* is an opportunistic, pathogenic fungus found in the human body. It lives on the skin and inside of the body, typically without any harm to the host. In immunocompromised individuals, *C. albicans* will exhibit a pathogenic response and cause harm in the form of vaginal yeast infections, oral thrush, candidiasis, and sepsis, to name a few. In freshwater drowning environments, *C. albicans* can also take advantage of the host, causing fungal contamination to the tissue. This contamination can affect the integrity of the tissue samples that are collected. Materials for biological testing are expensive and it is not cost effective to process potentially contaminated samples. Crime labs may choose not to process such samples due to assumed DNA degradation.

By inoculating tissue samples with a clinical wild-type strain (SC5314) of *C. albicans* in river water, Optical Density (OD) readings can be taken to evaluate the level of *C. albicans* growing in the presence of the tissue. DNA processing of the tissue will allow the integrity of the tissue to be evaluated. Tissue extraction via QIAamp® DNA Mini Kit will isolate the human DNA within the tissue sample. Quantitation by Quantifiler® HP DNA Quantitation Kit and QuantStudio™ 5 Real-Time PCR System will measure the amount of DNA extracted. Amplification by GlobalFiler® PCR Amplification Kit will make copies of the DNA sequence. Genotyping via SeqStudio™ Genetic Analyzer will allow the genetic differences between samples to be evaluated. Analysis by GeneMarker® HID of the data from the genetic analyzer will be able to compare genotypes between the samples. This overall process will allow for a better understanding of the integrity of the experimental tissue sample. The level of contamination may also help evaluate if it is positively or negatively correlated with the growth rate of the fungus. The results are expected to support the hypothesis that there is positive correlation between the two, that is that the more *C. albicans* that is present, the lesser the integrity of the DNA.

This work aims to give forensic laboratories a better understanding of tissue contamination as it relates to fungus and improve decision-making in regard to processing such samples.

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**Drowning, *Candida albicans*, Contamination**

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**Y2 The Production of Recombinant Semenogelin Protein Fragments for the Improved Antibody-Based Detection of Body Fluids**

Thomas Washington, BS\*, Duquesne University, Pittsburgh, PA; William Gibbs, Duquesne University, Pittsburgh, PA; Clancy McIntyre, Duquesne University, Pittsburgh, PA; Pamela Marshall, PhD, Duquesne University, Pittsburgh, PA; Michael Jensen-Seaman, PhD, Duquesne University, Pittsburgh, PA

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**Learning Overview:** After attending this presentation, attendees will have learned about recombinant protein expression and how to implement allelic variation considerations into forensic serology.

**Impact Statement:** This presentation will demonstrate one approach to providing an improved semen identification test less prone to false positives and negatives.

The identification of body fluids on evidence items is an important component of forensic science. Sometimes, simply identifying a body fluid is enough to impact a case.<sup>1</sup> Forensic serology is also critical because determining if a body fluid is present, then identifying it, allows for downstream laboratory testing, including DNA analysis.<sup>1</sup> A body fluid commonly discovered at crime scenes is semen. Targets of current forensic semen identification tests include the seminal enzymes Prostate-Specific Antigen (PSA) and Acid Phosphatase (AP), as well as spermatozoa and semenogelins. Proteins known as Semenogelin I (Sg I) and Semenogelin II (Sg II) are the main structural components of the gelatinous mass formed by human semen after ejaculation.<sup>2,3</sup> These proteins make up 20% to 40% of all seminal plasma protein.<sup>2,3</sup> The coagulum liquefies 5 to 20 minutes after ejaculation.<sup>2</sup> PSA plays a large role in this process, known as liquefaction, by cleaving semenogelin proteins through protease activity.<sup>2,3</sup> Current forensic semen identification tests have flaws, including false positives and false negatives. Therefore, this study sought to develop an improved semen identification test.

In order to accomplish this, this study produced and purified fragments of Sg I protein located between PSA cleavage sites.<sup>4</sup> Using databases of human genetic variation, this study found one such fragment of Sg I (later named the THW fragment) containing no common non-synonymous Single Nucleotide Polymorphisms (SNPs). This study Polymerase Chain Reaction (PCR) -amplified the THW fragment and used an *E. coli* expression system to produce THW protein. Affinity chromatography was used to purify THW protein. A different fragment of Sg I (the Seminal Plasma Motility Inhibitor [SPMI] fragment) targeted by a previously developed semenogelin-targeted test, and an allelic variant of the SPMI fragment containing a common non-synonymous SNP, were produced and purified using the same system used for THW.<sup>5</sup> This study evaluated the ability to detect these recombinant semenogelin protein fragments with a commercially available semenogelin-targeted semen identification kit.

Overall, the study seeks to improve serological testing aimed at semen identification by providing a test less prone to false positives and negatives, through targeting semenogelin and consideration of common allelic variants. By providing an improved test, the potential to solve more sexual assault cases is increased.

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2. Robert, M.; Gagnon, C. Semenogelin I: A coagulum forming, multifunctional seminal vesicle protein. *CMLS, Cell. Mol. Life Sci.* 1999, 55, 944-960.
3. de Lamirande, E. Semenogelin, the Main Protein of the Human Semen Coagulum, Regulates Sperm Function. *Semin Thromb Hemost* 2007, 33 (1), 60-68, DOI: 10.1055/s-2006-958463.
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**Semen Identification, Semenogelin, Recombinant Proteins**

### Y3 A New Algorithm for Source Identification of Look-Alike Footwear Impressions Based on Automatic Alignment

Hana Lee\*, Iowa State University and Center for Statistics and Applications in Forensic Evidence, Ames, IA; Alicia Carriquiry, PhD, Iowa State University and Center for Statistics and Applications in Forensic Evidence, Ames, IA

**Learning Overview:** After attending this presentation, attendees will have learned about automatically aligning two look-alike images of footwear impressions to the extent that human examiners would do prior to comparison. Moreover, this study demonstrates how well the proposed algorithm based on the features resulting from the alignment can identify whether the two images were taken from the same shoe or not. The images used were obtained from Everspy Outsole Scanner collected by the Center for Statistics and Applications in Forensic Evidence (CSAFE).

**Impact Statement:** Digital images of footwear impressions taken at a crime scene are often useful to connect a suspect to the crime scene. Comparing an image from a crime scene with one from a suspect's shoe has usually been conducted by manual alignment with Photoshop® and examiners' experience for comparison, which is time consuming and labor intensive. The proposed algorithm enables objective comparison of alike images of footwear impressions with high accuracy, in less than a minute per comparison. Therefore, this algorithm can be a useful tool for examiners. The automatic alignment process that is proposed produces images that are ready to be overlaid, and thus are convenient for further analysis.

Suppose that there are two images denoted by  $R$ , and  $M$  where  $R$  indicates a reference image, and  $M$  indicates a moving image that will be aligned to  $R$ . First, a thousand Speeded Up Robust Features (SURFs) are extracted from each of  $R$  and  $M$ . SURF is an interest point descriptor invariant to scale and rotation.<sup>1</sup> The strongest 500 SURFs of  $M$  are aligned to those of  $R$  by the Iterative Closest Point (ICP) algorithm, that minimizes the difference between two sets of points, by providing the optimized rotation angle and translation of the SURFs of  $M$ . Next, the similarity (or dissimilarity) features are computed using the aligned 500 SURFs of  $M$  and 1,000 SURFs of  $R$ . These include the closest distance, the number of  $R$ 's SURFs around the aligned SURFs of  $M$ , and the number of aligned SURFs of  $M$  that have no neighboring SURFs on  $R$ . Additionally, the maximum of the phase correlation between the transformed  $M$  and  $R$  is also used as a similarity feature. Phase correlation estimates the translative offset at which the inverse Fourier transform of the normalized cross spectrum between two images is maximized.<sup>2</sup> Note, therefore, that the alignment by SURF and ICP and the adjusted translation by maximum phase correlation can align images better because it considers the offset due to the transition from SURF points to pixels. Lastly, the similarity features are combined using a random forest to estimate the probability that  $M$  and  $R$  came from the same shoe.

The proposed algorithm's performance in terms of a Receiver Operating Characteristic (ROC) curve was almost comparable with 0.96 of Area Under the Curve (AUC) to Park et al. based on maximum cliques that produced AUC greater than 0.99 where two images from the same shoe are just replicated scans of the shoe, and those from different shoes have the same class characteristics.<sup>3</sup> On the other hand, when using two images from the same shoe taken several months apart so that they possess different wear and tear, the proposed algorithm with 0.94 of AUC outperformed Park et al. with 0.85 of AUC, which demonstrates its distinguishing ability in a more practical and challenging scenario.

#### Reference(s):

1. Bay, H.; Tuytelaars, T.; Van Gool, L. (2006, May). Surf: Speeded up robust features. In: *European conference on computer vision* (pp. 404-417). Springer, Berlin, Heidelberg.
2. Alba, A.; Aguilar-Ponce, R.M.; Viguera-Gómez, J.F.; Arce-Santana, E. (2012, October). Phase correlation based image alignment with subpixel accuracy. In: *Mexican International Conference on Artificial Intelligence* (pp. 171-182). Springer, Berlin, Heidelberg.
3. Park, S.; Carriquiry, A. (2020). An algorithm to compare two-dimensional footwear outsole images using maximum cliques and speeded-up robust feature. *Statistical Analysis and Data Mining: The ASA Data Science Journal*, 13(2), 188-199.

#### Algorithm for Source Identification, Automatic Alignment, Footwear Impressions

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## Y4 An Ensemble of Score-Based Likelihood Ratio (SLR) Systems for Forensic Evidence

Federico Veneri, MS\*, Iowa State University, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA

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**Learning Overview:** The objective of this presentation is to provide attendees with an introduction to the proposed approach to ensemble SLR systems. Using the elemental composition of glass, this presentation illustrates how sampling can be used to create sets of independent observations to estimate random forest-based similarity metrics and density estimations and ensemble their result into a final SLR.

**Impact Statement:** Despite their obvious drawbacks, SLRs continue to be the preferred method of quantification for complex impression and pattern evidence. This presentation aims to provide an improved methodology for producing SLRs for use by forensic practitioners.

SLRs have been used as an alternative to feature-based likelihood ratios when the construction of a probabilistic model becomes challenging or infeasible.<sup>1</sup> Although SLRs have been shown to provide an alternative way to present a numeric assessment of evidential strength, there are still concerns regarding their use in a forensic setting.<sup>2</sup>

The SLR approach requires two key components. First, developing a (dis)similarity score, and second, estimating the distribution of the scores under both prosecutor and defense propositions. This process relies on the construction of pairwise comparisons in both stages. Previous work addresses the dependency on the sets used for producing SLR, how introducing perturbation to the sets can lead the forensic examiner to different conclusions, particularly the sensitivity during the second stage.<sup>3</sup>

A second less-explored dependence structure is produced because forensic glass evidence can be thought to be generated by a hierarchical model.<sup>4</sup> When a pairwise comparison is made, the same source enters the comparison multiple times, undermining the independence assumption often required by the methods used for developing a (dis)similarity score and estimating their distribution.

This work introduces an ensemble method for creating an SLR system that aims to reduce sensitivity to perturbation in the samples and remove the dependence structure. This study creates independent subsets by sampling only one comparison from each source to create base SLR systems, and then ensemble them in a final system that can be used in a later stage.

### Reference(s):

1. Hal S. Stern. Statistical Issues in Forensic Science. *Annual Review of Statistics and Its Application* 4 (2017): 225–244.
2. Cedric Neumann; Madeline Ausdemore. Defence against the Modern Arts: The Curse of Statistics—Part II: “Score-Based Likelihood Ratios.” *Law, Probability and Risk* 19, no. 1 (March 1, 2020): 21–42, <https://doi.org/10.1093/lpr/mgaa006>.
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### Score Likelihood Ratios, Learning Algorithms, Glass Evidence

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**Y5 A Forensic Assessment of Morphed Audios Using Conventional and Modern Techniques of Speaker Identification**

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**Learning Overview:** This presentation will help identify if audio is edited using software. It can also help in identifying the challenges present in the current speaker identification techniques used in forensic science.

**Impact Statement:** This presentation will impact the forensic science community by helping identify if audio is edited using software and by identifying the challenges present in the current speaker identification techniques used in forensic science.

Perpetration of crime using digital media has witnessed unprecedented escalation and modification apropos to the technological advances across the globe. The multimedia forensics, and the audio forensics in particular, has also harnessed the innovative counter-technological potential to pinpoint the identifiable elements in any crime for the overall aid of the criminal justice system. While speaker recognition and tests of authenticity constitute the bulk of the work in the audio forensics domain, the possibilities of exact identification is being constantly complicated by the scope of digitally altering the voices or converting them into completely unrecognizable voices, popularly referred to as morphing. Morphing of digital voices or converted voices is generated by the software freely available in the cloud system. Criminals may like to use this disguising morphing technology for ulterior motives with the instinct of camouflaging their identity. The software used in creating these audios have pre-set algorithms that modify the voice into that of alien, monster, or cartoon, or they can change the gender of the speaker in the final audio output in addition to providing several other options and kinds of morphed conversions. These softwares are freely available and most of them are compatible to Microsoft® Windows or Android® operating systems.

Crime laboratories across the world have graduated to using automated technologies for processing the various issues related to audio examination, but unfortunately not yet for tackling the issue of morphing. There are no validated techniques that have been able to produce a favorable statistic and conclusively identify the speaker. A survey of published literature would also highlight the absence of much scientific work on tackling morphing. The present research is thus an attempt to get some breakthrough in this largely untraversed scientific challenge. To conduct this research, 100 speakers (50 male and 50 female) who can speak Hindi and English were randomly selected. The speakers were given the transcript (of approximately two minutes duration) 30 minutes before their audio sample collection. All speakers were asked to repeat the script five times with the a ten-minute gap in each recording. Every fourth recording of the speaker was used to create the morphed audio using one software each from Android®- and Microsoft® Windows-based operating systems. For the samples, analysis using auditory, spectrographic, and automatic techniques were used.

The results of this study clearly indicated that no single method is 100% capable for identification of morphed audios in the present time. But it was felt that the combination of these techniques can be useful when one is performing linguistic and prosodic feature analysis; for the type of phoneme, the formant frequency value (out of all of the four formants calculated) can be used for the identification. Also, while doing the analysis using automatic technique, the log<sub>10</sub> value was very low, and in most of the comparison, the concluding hypothesis indicated was “control and questioned audio do not have same source.” Certainly, with limitations, some specific formant frequencies indicative of the morphing can be combined with auditory parameters, especially the degradation of background noise for a suspected case of morphing, then comparing it with a possible list of suspects for speaker identification based on spectrographic analysis, which can help in recognizing the perpetrators of crime. Later, the audios were also compared on the basis of the American Board of Recorded Evidence, and the result for maximum speakers was in the inconclusive category.

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**Morphing, Speaker Identification, Digital Audio**

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**Y6    Toward a Score-Based Likelihood Ratio (SLR) for Footwear Analysis**

*Valerie Han, BS\*, Iowa State University, Ames, IA; Alicia Carriquiry, PhD, Iowa State University, Ames, IA*

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**Learning Overview:** By attending this presentation, attendees will gain a better understanding of how to formulate an SLR on multivariate features and the impact of non-independence of observations.

**Impact Statement:** A shoeprint is found at a crime scene and investigators want to determine whether the shoeprint matches the shoeprint made by the shoe of a person of interest. This presentation will impact the forensic science community by explaining how SLRs can be computed to quantify the strength of the evidence in this scenario. This presentation will also explore how the dependence of the observations affect the estimation of the SLR.

SLRs are a statistical method used to quantify the weight of evidence and have been used in many areas of forensics, including footwear analysis. Park and Carriquiry proposed a method based on the idea of a maximal clique to find the best alignment for a pair of shoeprint images.<sup>1</sup> Once two images are aligned, variables can be measured that may help quantify the closeness between the aligned images. Three variables are considered: maximal clique size, percent of overlapping features in the two images, and median Euclidean distance between overlapping features. The scores on their own are difficult to interpret, so they must then be compared with two different reference sets of scores: matching and non-matching. Matching scores are computed from features of a pair of shoeprints known to come from the same shoe. Non-matching scores are computed from two shoeprints known to come from two different shoes.

Computing the SLR requires estimating the distribution of the three variables. Since the distribution is not a recognizable parametric distribution, Kernel Density Estimation (KDE) is used. However, since the three variables are computed from pairs of shoes, the observations are dependent. This present research explores how dependent observations affect the kernel density estimation and thus the SLR, and three different ways to modify the KDE are proposed and evaluated by measuring the accuracy and discriminating power of the estimated SLRs while also measuring the calibration, generalization, coherence, and robustness.<sup>2</sup>

**Reference(s):**

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- <sup>2.</sup> Ramos, Daniel et al. Validation of forensic automatic likelihood ratio methods. *Handbook of Forensic Statistics*. Chapman and Hall/CRC, 2020. 143-162.

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**Footwear Evidence, Score-Based Likelihood Ratios, Weight of Evidence**



**Y7 The Development of a Rapid Drug Detection Method for Insects Using Paper Spray Ionization Mass Spectrometry (PSI-MS)**

*Alexandria Plyler, BS\*, Duquesne University, Pittsburgh, PA*

**Learning Overview:** Attendees can expect to learn about a toxicological method that, per research, contains a novel combination of methods and instrumentation. Forensic entomotoxicology is still a relatively underdeveloped field, making its study in an academic setting important should it ever be implemented in the field.

**Impact Statement:** PSI-MS as an analytical technique offers great advantages to not only forensic toxicology, but to forensic chemistry and trace evidence professions as well. Its ability to detect analytes with minimal sample preparation yet without sacrificing sensitivity or specificity can make it a valuable tool by which crime labs can combat the evidence backlog.

Insects have the ability to reflect the chemical environment in which they have developed. Thus, necrogenous insects can reflect the chemical environments of the cadavers they have fed on. In cases where cadavers are skeletonized, burned, or otherwise too badly damaged for tissues to be collected, forensic scientists can potentially utilize insect larvae as an alternate toxicological matrix to detect drugs in cadavers. Traditional techniques for drug detection by Gas Chromatography/Mass Spectrometry (GC/MS) and/or Liquid Chromatography/Mass Spectrometry (LC/MS) often involve lengthy sample preparation, use expensive reagents, and are destructive to important evidence. This poses a problem for crime labs that do not possess the time, funding, or resources to perform such techniques.

Analysis of insects using PSI-MS, an analytical method requiring little to no sample preparation, could provide a rapid, cost-effective, and non-destructive alternative form of toxicological analysis. PSI-MS has the distinct advantage of being an ambient ionization technique, allowing for the running of insect samples with virtually no sample preparation at STP without sacrificing sensitivity and specificity. In addition, the use of disposable paper triangles during PSI-MS analysis greatly reduces sample carryover between runs.

To validate a PSI-MS method for insects, several different extraction techniques for drug detection in the model insect *T. molitor* were explored. *T. molitor* larvae first fed on substrate spiked with Phenethylamine (PEA), a surrogate for illicit monoamine drugs such as methamphetamine and MDMA. Various extraction techniques were then explored, including ethanol extraction, methanol extraction, and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction. In addition, a novel “bug-spray” technique which bypassed insect sample preparation entirely was investigated. By employing each extraction method and analyzing the insect samples using PSI-MS with Collision-Induced Dissociation (CID), the most time-efficient, cost-effective, and reliable detection method was developed. The successful implementation of PSI-MS in a forensic setting can pose a useful tool for combating the evidence backlog due to its rapid nature. The ability to study the chemical content of an insect’s environment could have significant potential not only in forensic science, but in conservation studies and the environmental sciences as well.

**Reference(s):**

1. Bugelli, V.; Papi, L.; Fornaro, S.; Stefanelli, F.; Chericoni, S.; Giusiani, M.; Vanin, S.; Campobasso, C.P. Entomotoxicology in burnt bodies: A case of maternal filicide-suicide by fire. *Int J Legal Med* 2017, 131 (5), 1299-1306, DOI: 10.1007/s00414-017-1628-0.
2. Manhoff, D.T.; Hood, I.; Caputo, F.; Perry, J.; Rosen, S.; Mirchandani, H.G. Cocaine in Decomposed Human Remains. *Journal of Forensic Sciences* 1991, 36 (6), 1732-1735.
3. McBride, E.M.; Mach, P.M.; Dhummakupt, E.S.; Dowling, S.; Carmany, D.O.; Demond, P.S.; Rizzo, G.; Manicke, N.E.; Glaros, T. Paper spray ionization: Applications and perspectives. *TrAC Trends in Analytical Chemistry* 2019, 118, 722-730, DOI: 10.1016/j.trac.2019.06.028.

**Entomotoxicology, Paper Spray Ionization, Mass Spectrometry**

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**Y8 A Characterization of Metal-Peroxide Explosive Complexes Using Paper Spray Ionization-Tandem Mass Spectrometry (PSI-MS/MS)**

*Kayla Massari\*, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** After attending this presentation, attendees will better understand how the analysis of metal-peroxide complexes using PSI-MS/MS allows the presence of these complexes to be reliably detected in pre- and post-blast explosive residue.

**Impact Statement:** Organic peroxides have become a common agent in Improvised Explosive Devices (IEDs) because they are accessible for purchase and are susceptible to extremely exothermic reactions due to the instability of the peroxide bond.<sup>1</sup> IEDs have been used in acts of terrorism such as the Oklahoma City bombing and the Olympic Park bombing, and the detection of the explosive component is necessary in terms of eventually identifying the perpetrator who had the means and materials to make the IED.<sup>2</sup> Thus, a reliable and rapid detection method for organic peroxides commonly used in IEDs is of interest for the analysis of pre- and post-blast residue in forensic investigations.

The goal of this project is to develop PSI-MS/MS with Collision-Induced Dissociation (CID) for the detection and identification of peroxide-based explosives. Benzoyl peroxide and dicumyl peroxide were used as model compounds. To complement experimental studies, Density Functional Theory (DFT) was used to determine probable ion structures. Benzoyl peroxide and dicumyl peroxide samples were spiked with lithium, sodium, and potassium alkali metal cations. The peroxides were also pipetted onto silver-impregnated filter paper. These samples were ionized utilizing the THERMO FISHER™ LTQ-XL, and the fragmentation patterns of the peroxide-metal complexes were investigated using CID at the MS<sup>2</sup> stage. DFT calculations of ion structures were performed at the B3LYP/6-311+G(d) and B3LYP/MWB28/6-311+G(d) level of theory.

Initial experiments showed that both dicumyl and benzoyl peroxide form abundant complexes with Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup> and Ag<sup>+</sup> by PSI. In general, PSI produced 1:1 complexes between the peroxides and Li<sup>+</sup>, Na<sup>+</sup>, and K<sup>+</sup>. Studies completed with silver cations and both model peroxides showed that PSI will generate Ag<sup>+</sup> complexes with 1 or 2 peroxide ligands. Because the silver-impregnated filter paper allowed for the Ag-peroxide complexes to form, it was determined that a swipe sampling method should be tested with the other metal-peroxide complexes. If this method is successful, it would allow a sample to be swiped from a surface using filter paper and analyzed using PSI with minimal sample preparation.

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**Mass Spectrometry, IED, Peroxide-Based Explosives**

**Y9 The Extraction and Identification of Illicit Compounds From Baked Goods Using Paper Spray Ionization-Tandem Mass Spectrometry**

*Isabella Haberstock\*, Duquesne University, Pittsburgh, PA*

**Learning Overview:** After attending this presentation, attendees will better understand how the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) solid phase extraction method can be used to extract an illicit compound from a complex matrix containing proteins, carbohydrates, and lipids. Attendees will also learn about the versatility of paper spray ionization-mass spectrometry in the rapid analysis of the QuEChERS supernatant for the presence of the illicit compound.

**Impact Statement:** Complex matrices like cookies and other baked goods have become common methods of consumption for illicit substances, and a rapid and accurate method for the extraction and detection of these substances is necessary for ongoing forensic investigations. Forensic toxicological analysis in drug-related cases often involves the detection of substances like MDMA, amphetamine, and methamphetamine, so an efficient solid phase extraction method would benefit time-sensitive investigations.<sup>1</sup> The paper spray ionization method can also be optimized and has the potential to advance the field of drug detection because of its versatility and rapidity.<sup>2</sup>

The goal of this project is to couple the QuEChERS extraction method with paper spray ionization-mass spectrometry to create an optimized extraction and detection method for illicit substances in baked goods. The QuEChERS method, although not typically used for baked goods, isolates the illicit compound from a complex matrix of carbohydrates, lipids, and proteins, allowing for accurate detection of the compound.<sup>3</sup> Paper spray ionization is a versatile method that produces accurate quantitative and qualitative data for drug detection studies.<sup>4</sup> Dough for individual sugar cookies was made using a basic recipe, and the cookies were spiked with 0 $\mu$ L, 1 $\mu$ L, 3 $\mu$ L, 5 $\mu$ L, and 10 $\mu$ L of phenethylamine. Each cookie was extracted in acetonitrile, magnesium sulfate, sodium chloride, and a lipid sorbent for the QuEChERS method.<sup>3</sup> The supernatant was analyzed using the THERMO FISHER™ LTQ-XL for the presence of the phenethylamine peak, which was confirmed through the use of tandem mass spectrometry and Collision-Induced Dissociation (CID). Using paper spray ionization-mass spectrometry, the phenethylamine peak was seen in the 1 $\mu$ L, 3 $\mu$ L, 5 $\mu$ L, and 10 $\mu$ L phenethylamine cookies. CID on the phenethylamine peak confirmed these results for each cookie. The cookie with 0 $\mu$ L phenethylamine did not display the phenethylamine peak, which confirmed that the cookies were not cross contaminated. Because phenethylamine was successfully extracted and detected using QuEChERS and paper spray ionization, future experiments include the quantification of the illicit substance, the use of MDMA in place of phenethylamine, and the use of the Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) to further perform targeted analysis on the extracted illicit substance.

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**Paper Spray Ionization-Mass Spectrometry, QuEChERS Extraction, Baked Edibles**

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**Y10 Estimating Decomposition Time Via Bacteria From Rat Models**

*Kayce Boggess, BS\*, Duquesne University, Pittsburgh, PA; Jan Janecka, PhD, Duquesne University, Pittsburgh, PA; Lisa Ludvico, PhD, Duquesne University, Pittsburgh, PA; Evan Penrod, MS, Duquesne University, Pittsburgh, PA; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** By attending this presentation, attendees will learn more about potential bacterial patterns and abundance when looking at necro-microbiomes that could be implemented in Postmortem Interval (PMI) estimations.

**Impact Statement:** PMI can be an invaluable asset to crime scene investigators when investigating a crime involving a dead body. It can help to establish a time frame of death to prove or disprove certain causes of death. It could even change a guilty verdict. However, PMI can be hard to estimate due to several factors such as insect activity, temperature, and where the body is located at death. These elements can affect the rate of decomposition, making it nearly impossible to find out the time frame of death. In these situations, the only decomposition methods would be only the body itself, which includes its enzymes and microbiome. Thus, by incorporating bacterial analysis into PMI calculations, a better time frame of death could be found in these rarer instances. A bacterial pattern of abundance could spell out how long the person has been deceased and could help bring truth to a case.

This study strives to learn whether a microbiome can be used in closed-environment cases to estimate decomposition time. Microbiomes are communities of microbes that live on and in human bodies. Through their unique make-up per individual, identifying characteristics can be discovered by analyzing the organisms present. Current research looks at an open-air environment that considers the amount of decomposition to determine a time of death. In closed-air cases, however, a victim may decompose differently, limiting the ability to estimate time of death. This can lead decomposition to look completely different in closed environments when only the microbiome and other bacterial species are present to perform decomposition. Thus, with these closed-air cases, it is difficult to use traditional means to determine the PMI.

The purpose of this study is to use the microbiome's abundance and pattern to potentially determine a time of death based on decomposition time to be used in closed-air cases. This study utilizes a week-long trial of having 40 rats decomposing, with half in an open-air setup and the other half in a closed-air setup. The rats were swabbed every other day. By using Dneasy® PowerSoil Pro Kit DNA extraction on the bacterial species and MiSeq® Illumina® Next-Generation Sequencing or Massively Parallel Sequencing (MPS) on the swabs, this study aims to find a common pattern of decomposition between different rat models to better estimate time of death. This data will be processed downstream utilizing QIIME2™ and Weka to create a taxonomic bar graph and a decision tree, respectfully. These tools can be utilized to estimate not only the breakdown of the bacterial organisms from QIIME2™, but are also a way for a computer to estimate time of death from the patterns inputted from Weka. By using this research's data and a literature review of previous studies exploring necro-microbiomes, a more accurate PMI can be established. The literature review so far has shown that the percentage difference between the main four phyla, Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, could allude to some pattern. Further review of the literature and this study's trial will be compared to come to a true conclusion due to more data being available to be placed into QIIME2™ and Weka.

This study concludes if there were a pattern and/or order of bacterium in microbiomes that relate to decomposition time and if there is a major difference in this information between closed and open-air environments. Per research, there is not another study that examines closed-environment decomposition in relation to bacterial activity. Thus, this study will be the first to discover a way to use microbiomes to estimate the time of death in closed-environment cases, thus providing a more accurate time of death that can potentially be utilized in these certain forensic cases, allowing a narrowing time window for alibis or to search for evidence. The knowledge gains from this research about microbiome examination could also be applied to forensic cases that may not normally have a time of death or even for cases that may have been too broad in their original PMI category. This impact to the forensic community will increase the accuracy of PMI in both open-air and closed-air cases.

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**Microbiome, Decomposition Time, Postmortem Interval (PMI)**

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### Y11 The Identification of Counterfeit Alcohols in Cocktails by Paper Spray Ionization-Mass Spectrometry (PSI-MS)

*Derek Heacox, BS\*, Duquesne University, Pittsburgh, PA; Michael Van Stipdonk, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Overview:** After attending this presentation, attendees will understand the origin of methanol in alcoholic beverages, how PSI-MS can be utilized in detecting laced alcohol, and what can be done if an alcoholic beverage is suspected to be tainted.

**Impact Statement:** This presentation will provide a greater understanding of the methods of detecting methanol in a suspected tainted alcohol case.

In May of 2020, as a result of the SARS-CoV-2 virus, distilleries in Chiconautla, Mexico, were labeled non-essential, resulting in an influx of counterfeit liquors by criminal organizations. Twenty people in Chiconautla died as a result of consuming the counterfeit liquor. As of April 2021, 326 people have been treated with methanol poisoning and 127 people in the Dominican Republic have died as a result of consuming methanol-laced alcoholic beverages. The consumption of counterfeit alcohol is a worldwide problem and developing a method to the legitimacy of alcohol consumed could save thousands of lives yearly. Thirty milliliters of methanol was determined to be the amount of methanol, when consumed, to cause severe illness, such as blindness or death. The purpose of this study is to determine if a counterfeit alcohol can be found in a cocktail-like solution using PSI-MS. A triangular piece of filter paper was infused with silver nitrate, and a series of deuterated ethanol and methanol standards were created to determine if methanol could be found using the PSI-MS methods. A vodka soda sample was then made containing a lethal amount of methanol, Pinnacle® vodka, and Sprite®. In the pure Pinnacle® vodka sample, only trace amounts of methanol were found. During the distillation process, 100% of the methanol cannot be removed, but trace amounts of methanol can be passed through the body without harm. During a home distillation, methanol is cut in to the final product to increase the overall yield; this was a main contributor to methanol poisoning. Standard experiments have been conducted to determine the limit of detection of the PSI-MS. Methanol-d4 had been utilized to determine the detection limit of the instrument.

Further experiments will be conducted to determine if quantification of ethanol and methanol can be determined using the PSI-MS method. This will be used to aid in the determination of whether a person had enough methanol in their system to cause dizziness, illness, or death. Methanol is a volatile liquid; a time trial will be conducted in order to ascertain a time limitation at which the methanol would be detected. Methanol that had been present on a filter paper minutes or hours after preparation are to be expected to be detected. This would lead to further studies to create a litmus test for methanol-laced beverages. A consumer could dip their drink on a testing paper and a color change would indicate trace or potentially lethal amounts of methanol in their beverage.

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**Forensic Science, Paper Spray Ionization, Counterfeit Alcohol**

**Y12 The Analysis of Commercially Available Kratom Products in Richmond, Virginia, Using Direct Analysis in Real Time-Mass Spectrometry (DART®-MS) and Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES)**

*James Fleming, BS, Virginia Commonwealth University, Richmond, VA; Emanuele Alves, PhD, Virginia Commonwealth University, Richmond, VA*

**Learning Overview:** After attending this presentation, participants will have learned about the inorganic and organic chemical profile of kratom products and the methods used for the detection of adulterants and/or heavy metals. In addition, participants will learn the fundamentals of Direct Analysis in Real Time (DART®) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and how it can be used in forensic casework.

**Impact Statement:** This presentation will impact the forensic science community by informing participants about the variability of active compounds and heavy metals present in kratom products that show toxicological and public health concerns. This work highlights the need to “surpass the challenges of a modern forensic science world”, especially as kratom usage is evolving.

Kratom, also known as “Ketum” or “Biak-biak”, is a novel psychoactive substance that has gained popularity in the United States within the past ten years. Kratom originates from the leaves of the *Mitragyna speciosa* tree that is native to Southeast Asia, where it is known for its historical, medical, and recreational uses. Today, a variety of kratom products are commercially available in the United States, including powder, tea, capsules, extracts, and even carbonated beverages. These products are sold on the Internet, in tobacco shops, and in convenience stores. Mitragynine is the main psychoactive substance in kratom, followed by its derivative 7-hydroxymitragynine, which both play a key role in kratom central nervous system effects<sup>1</sup>. As a natural product, kratom is a complex mixture of more than 40 alkaloids with different properties. In excessive amounts, kratom leaves can cause stupor, sweating, dizziness, nausea, and dysphoria – effects that are usually expressed in opioid overdose.

Kratom products are not regulated and products often include a statement of “not for human consumption” on the labels. “Krypton” and “4x100” are kratom cocktails previously associated to fatal cases. “Krypton” combines kratom leaves with O-desmethyltramadol, while “4x100” combines kratom leaves with Coca-Cola and codeine containing cough syrup<sup>2</sup>. Due to the lack of regulation of commercial products, lethal cocktails like these demonstrate the need for a quick and easy method to detect unlisted components in commercially available kratom products. The Food and Drug Administration (FDA) has found toxic levels of lead and nickel in various kratom products. Furthermore, several studies have described higher concentration of mitragynine and other alkaloids in different commercial products obtained from kratom.

For the chemical profile study, 29 kratom samples were obtained from tobacco and vape shops in the Richmond, Virginia area. Samples included powders, teas, capsules, extracts, and a carbonated beverage. Active compounds were analyzed using Direct Analysis in Real Time-Time of Flight-Mass Spectrometry (DART-MS) and elements were analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The DART analysis was performed for the untargeted screening of the exact masses of kratom alkaloids. For this analysis, a capillary tube was dipped in a methanolic solution obtained from kratom samples and introduced into the helium stream of the DART-MS. Elements included Al, As, Cu, Fe, K, Mg, Mn, Na, Ni, and Pb. For ICP-OES analysis, powder samples underwent an acid digestion.

Kratom use has become more popular with each passing year, and while methods for kratom active compounds already exist, there is a no information about the total chemical profile of samples currently in the market. As a herbal product, no quality control is expected from manufacturers, which brings a public health concern due to the association of high concentrations of active substances and toxic metal levels in commercial kratom samples.

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**Kratom, DART®, ICP/OES**

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- Candelario-Gorbea, Mariel-H45  
ANDE (Discussion of Commercial Products or Services)  
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Victims of Crime Assistance (Grant Support)
- Canellas, Marc-F5  
STRmix, DNA Software (Discussion of Commercial Products or Services)
- Cantu, Cesar-B7  
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- Cappas, Veronica-B185, H114  
American Type Culture Collection (Discussion of Commercial Products or Services)  
The Pennsylvania State University (Stipend)  
Copan, Promega, SoftGenetics LLC, Applied Biosystems (Discussion of Commercial Products or Services)  
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- Carrillo, Kayli-B109  
Cadre Forensics (Discussion of Commercial Products or Services)
- Carrquiry, Alicia-E125  
National Institute of Standards and Technology and Iowa State University (Grant Support and Stipend)
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LODOX Imaging Scanners (Discussion of Commercial Products or Services)
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Zoom Video Communications (Discussion of Commercial Products or Services)
- Cerrito, Paola-G17  
National Science Foundation (Grant Support)
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National Institute of Justice, NEAFS (Grant Support)
- Chaski, Carole-D8, D12  
ALIAS Technology LLC (Discussion of Commercial Products or Services)
- Cheng, Kevin-B139, B143  
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- Christy, Brenda-W3  
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- Claasen, Brittany-B182  
Cedar Crest College (Stipend)
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Foster & Freeman, Ltd. (Discussion of Commercial Products or Services)

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Open Source and/or free to use software such as Proxmox, Open Media Vault, Docker and Portainer (Discussion of Commercial Products or Services)

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National Institute of Justice (Grant Support)

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Micrologix 1400, Micrologix 1100, Micro 820, Micro 850, CLICK Koyo, Schneider Electric Modicon M221 (Discussion of Commercial Products or Services)

## R

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